AD-A043 877 JOHNS HOPKINS UNIV BALTIMORE MD SCHOOL OF HYGIENE A--ETC F/G 6/15

CHEMOTHERAPY OF SCHISTOSOMIASIS.(U)

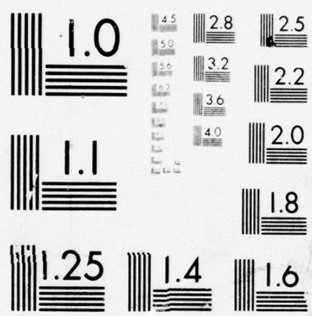
OCT 76 E BUEDING DAMD17-74-C-4009

UNCLASSIFIED NL

| OF |

AD
A043877

END
DATE
FILMED
9-77
DDC



MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

SCHOOL OF HYGIENE AND PUBLIC HEALTH
Department of Pathobiology
THE JOHNS HOPKINS UNIVERSITY
615 N. Wolfe Street
Baltimore, Maryland 21205

12
B.S.

FINAL REPORT

U. S. Army Medical Research and Development Command
Contract DAMD-17-74-C-4009

Project Title: Chemotherapy of Schistosomiasis

Inclusive dates: September 1, 1973, to
April 30, 1976

Principal Investigator: Ernest Bueding, M.D.

October 28, 1976

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) 6 Chemotherapy of Schistosomiasis		5. TYPE OF REPORT & PERIOD COVERED 9 Final Report, 1 September 1973 - 30 April 1976
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) 10 Ernest Bueding M.D.		8. CONTRACT OR GRANT NUMBER(s) 15 DAMD 17-74-C-4009
9. PERFORMING ORGANIZATION NAME AND ADDRESS The Johns Hopkins University School of Hygiene and Public Health Baltimore, Maryland 21218		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 16 62759A 3A762759A831 00.090
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Washington, D.C. 20314		12. REPORT DATE October 28, 1976
		13. NUMBER OF PAGES 61 17 01
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) 11 28 Oct 76		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

12 61p.

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Schistosomiasis, antischistosomal drugs, nitrovinylfurans, mutagenic effects of drugs, hycanthone, hycanthone analogs, IA-3 N-oxide, IA-4 N-oxide, drug metabolism, metrifonate, oxamniquine, hycanthone resistance.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Summary on reverse side.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

191 850

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SUMMARY

1. A formulation for enhancing the bioavailability and chemotherapeutic activity of an antischistosomal nitrovinylfuran has been developed.

2. In collaboration with Drs. Michael Cary and David Henry -- (Stanford Research Institute, the structure - antischistosomal activity relationship were investigating using over 100 newly synthesized compounds belonging to a single class, i.e., nitrovinylfurans.

3. Mutagenic and antischistosomal activities have been dissociated from hycanthone by suitable structuralterations. (Recently, two of the compounds, IA-3 and IA-4 Oxide , developed ~~by us~~ under this contract, have been investigated further at WRAMRI and found highly effective in the treatment of primates infected with Schistosoma mansoni.

4. Three metabolites of the most effective and least toxic antischistosomal hycanthone analog have been isolated and identified as the aldehyde, the desethyl aldehyde and the desethyl derivatives.

5. The mutagenic activities of five antischistosomal compounds have been determined and compared with each other. Three different procedures, one direct and two host mediated systems were used. Hycanthone was found to have kg for the highest mutagenic activity. The mutagenic activities of metrifonate, IA-4, oxamniquine and of IA-4 N-oxide were several orders of magnitude lower although schistosomicidal activities were approximately the same. Hence, structural modifications of the hycanthone molecule have reduced markedly mutagenic properties while desired chemotherapeutic activities have been maintained.

6. Genetically transferred resistance to the antischistosomal drug hycanthone can be produced in some strains of Schistosoma mansoni (a) by administration of the drug to hosts of adult schistosomes (Type I), (b) by exposure of immature schistosomes to the drug within their host, 27 to 30 days after infection with cercariae (Type II), and (c) by infection of the host with one sex of cercariae followed by infection with the opposite sex 13 to 133 days thereafter (Type III).

1. Attempts to develop formulations designed to increase the effectiveness of SQ 18, 506.

Previous studies have shown that two methods enhance considerably the bioavailability of SQ 18, 506: (a) reduction of the particle size below 4 microns (surface diameter) by ball-milling the compound in 25% glycerol containing a low concentration of a non-ionic detergent ("Cremophor El") for two weeks; (b) emulsification of this ball-milled preparation with a related non-ionic detergent ("Cremophor RH-40").

We have developed a new milling procedure which involves high speed grinding with sucrose crystals as an abrasive. This technique provides equivalent results with processing times of hours rather than weeks. Further testing is required to determine whether this new method can yield a particle size even more effective than the ball-milled product.

Previous observations suggested that conditions favoring the reduction by the host of the nitro group of SQ 18, 506 to a nitroso or hydroxylamino derivative enhances the antischistosomal activity of orally administered SQ 18, 506. Therefore, an attempt was made to determine whether a simultaneous supply to the host of reducing equivalents might not increase this reduction. Two sulfhydryl compounds - glutathione and crysteamine - were tested. As shown in Table I, pretreatment with, and coadministration of, these sulfhydryl compounds indeed increased significantly the antischistosomal effectiveness of SQ 18, 506 *in vivo*.

TABLE I

Effect of pretreatment with, and coadministration of, SH compounds on the antischistosomal activity of SQ 18, 506 in mice infected with *S. mansoni*

3 doses of SQ 18, 506 (ball milled and suspended in 20% Cremophor RH-40) were administered by gastric intubation at intervals of 8 and 16 hours. SH compounds were administered parenterally 16 hrs prior to the first dose of SQ 18, 506 and then simultaneously with this compound.

Expt. #.	Unit dose of SQ 18, 506 mg/kg	SH compd.	Dose of SH compd. mg/kg	# of mice	% reduction in worm numbers	% parasito cures
1	180	----	----	14	89	50
		glutathione	4x400 i. p.	15	100	100
2	170	----	----	14	68	14
		cysteamine	4x180 i. m.	15	98	87
3	160	----	----	16	63	12. 5
		glutathione	4x400 i. p.	15	100	100
4	145	----	----	13	48	0
		glutathione	4x400 i. p.	14	84	43
5	130	----	----	15	0	0
		cysteamine	4x180 i. m.	16	73	12. 5

2. Antischistosomal activity of other nitrovinylfurans

On the basis of the structural and conformational features common to those nitrofurans which exhibit schistosomicidal properties, a collaborative effort with Dr. David Henry and Michael Cory of the Stanford Research Institute was established. This project consisted in the design, synthesis and biological evaluation of over 100 nitrovinylfuranes and related compounds. These studies led to further elucidation of those structural features favorable to antischistosomal activity. The results of these studies are reported in Table II.

Table II

7 1

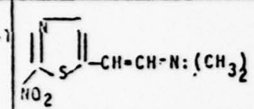
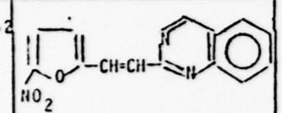
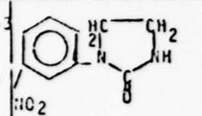
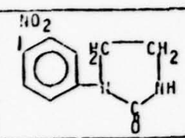
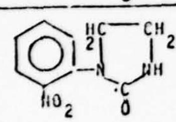
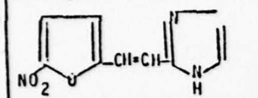
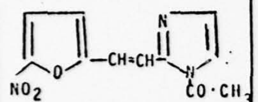
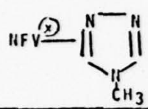
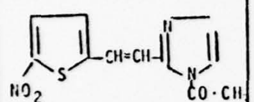
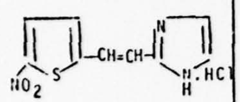
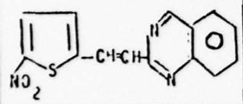
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
9535	H-1		II,2-3 II,152	1 Gm/kg once daily 5 days	0	0	0	3d.:43 6d.:67 15d.:20	0	0	0
0291	H-2 ✓		III,78	250 mg/kg once daily	0	0	0	3d.:57 4d.:36 5d.:37 13d.:18	0	0	0
0273	H-3 ✓		III,79	500 mg/kg b.i.d. for 1 day, followed by 250 mg/kg b.i.d. for 4 days	40	0	0	3d.:45 6d.:30	0	0	0
0275	H-4 ✓		III, 80	250 mg/kg b.i.d. for 5 days	0	0	11	3d.:47 4d.:40 13d.:15	0	0	0
0290	H-5 ✓		IV,43	250 mg/kg b.i.d. for 5 days	60	0	0	N;D.	0	0	0

Table II - continued

SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effects (4-5 weeks): reduction in # of worms	parasitol. cures
0429 I	H-6 I ✓		VI, 226 IV, 115	200 mg/kg b.i.d. 10 doses	0	46	37	71	50	21	0
			III, 250	400 mg/kg b.i.d. 8 doses	60	78	63	N.D.	95	68	25
0428 II	H-7 II ✓		III, 234	200 mg/kg b.i.d. 10 doses	0	34	46	N.D.	25	26	0
0450	H-8 ✓		III, 277	200 mg/kg b.i.d. 9 doses	70	77	84	80	100	N.D.: all surviving mice used.	
			II, 242	233 mg/kg b.i.d. 10 doses	25					S	C
0451	H-9 ✓		III, 284	200 mg/kg b.i.d. 10 doses	0	15	11	13	22	14	0
0452	H-10 ✓		III, 285	200 mg/kg b.i.d. 10 doses	20	22	15	17	15	0	0
~~0427~~ 0453	H-11 ✓		III, 286 IV, 21	200 mg/kg b.i.d. 10 doses	0	20	22	15	11	10	0

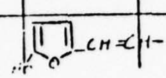
(x) Nitrofurylvinyl: 

Table II - continued

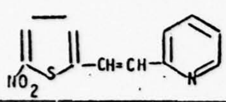
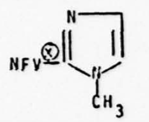
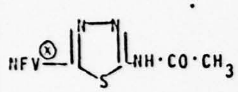
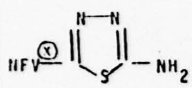
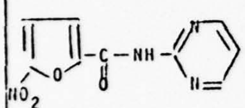
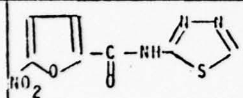
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
0454	H-12 ✓		III, 287	200 mg/kg b.i.d. 10 doses	0	21	28	50	25	19	0
0467 III	H-13 III ✓		IV, 36	200 mg/kg b.i.d. 10 doses	0	86	82	46	95	78	50
			IV, 95	200 mg/kg b.i.d. 10 doses	10	81	85	N.D.	95	85	44
			IV, 74	100 mg/kg b.i.d. 10 doses	0	42	46	32	20	21	0
0468	H-14 ✓		IV, 11	200 mg/kg b.i.d. 10 doses	0	22	11	13	20	8	0
0469 IV	H-15 IV ✓		IV, 12	200 mg/kg b.i.d. 10 doses	0	73	80	95	90	86	50
			IV, 113	200 mg/kg b.i.d. 10 doses	0	N.D.	N.D.	N.D.	N.D.	42	0
0470	H-16 ✓		IV, 13	200 mg/kg b.i.d. 10 doses	0	0	0	7	0	0	0
0471	H-17 ✓		IV, 22	200 mg/kg b.i.d. 10 doses	0	8	11	17	0	9	0

Table II - continued

7

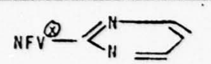
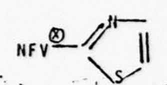
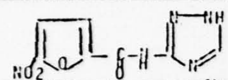
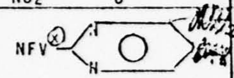
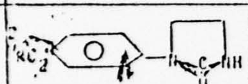
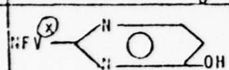
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
0472 V	H-18 V ✓		IV, 37	200 mg/kg b.i.d. 10 doses	0	91	86	100	100	100	100
			IV, 35	100 mg/kg b.i.d. 10 doses	0	53	50	93	35	18	0
0490 VI	H-19 VI ✓		IV, 70	250 mg/kg b.i.d. 10 doses	0	77	69	83	95	100	100
			IV, 175	250 mg/kg b.i.d. 10 doses	10	N.D.	N.D.	N.D.	N.D.	100	100
		[illegible]	IV, 174	250 mg/kg b.i.d. 8 doses	10	N.D.	N.D.	N.D.	N.D.	100	100
0489 VII	H-20 VII ✓		IV, 71	250 mg/kg b.i.d. 10 doses	0	70	56	97	75	91	20
[illegible]	H-21 ✓		IV, 68	250 mg/kg b.i.d. 10 doses	10	0	0	18	10	0	0
0496	H-22 ✓		IV, 89	200 mg/kg b.i.d. 10 doses	10	53	75	89	25	0	0
0498	H-23		IV, 90	200 mg/kg b.i.d. 10 doses	50	0	0	27	0	0	0
0726	H-24 ✓		IV, 160	250 mg/kg b.i.d. 4 doses 125 mg/kg 1 dose	80	26	30	33	10	N.D. surviving mice used for short time effects	
0727	H-25 ✓		IV, 165	250 mg/kg b.i.d. 10 doses	50	0	0	13	0	0	0

Table II - continued

5

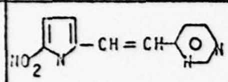
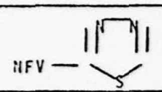
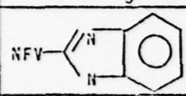
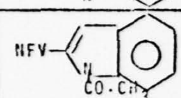
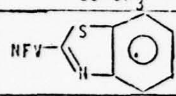
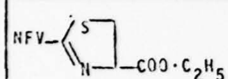
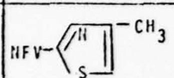
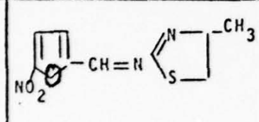
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
0738	H-26		IV, 179	250 mg/kg b.i.d. 10 doses	0	0	0	55	0	0	0
0739	H-27		IV, 180	250 mg/kg b.i.d. 10 doses	30	61	69	83	90	66	0 2T
0783	H-28		IV, 220	250 mg/kg b.i.d. 7 doses 125 mg/kg b.i.d. 1 dose	50	58	74	73	75	N.D.	2T N.D.
0732	H-29		IV, 222	250 mg/kg b.i.d. 7 doses 125 mg/kg b.i.d. 3 doses	25	59	76	80	60	19	0 2T
C784	H-30		IV, 223	250 mg/kg b.i.d. 10 doses	0	0	0	7	0	0	0
0735	H-31		IV, 235	250 mg/kg b.i.d. 10 doses	0	0	0	27	0	0	0
0787	H-32		IV, 241 +265	250 mg/kg b.i.d. 10 doses	0	62	58	92	80	19	40
0788	H-33		IV, 255 V, 108	250 mg/kg b.i.d. 10 doses 300 mg/kg b.i.d. 10 doses	0 0	0 0	2 0	0 0	0 0	63 25	0 0

Table II - continued

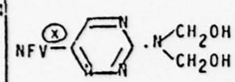
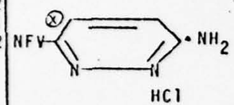
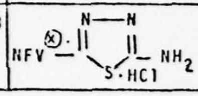
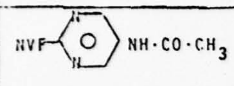
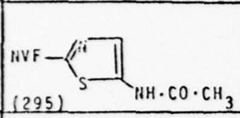
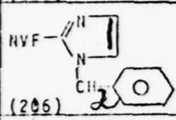
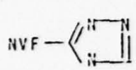
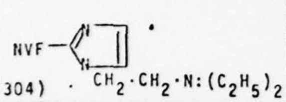
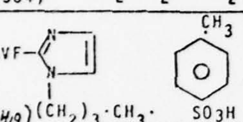
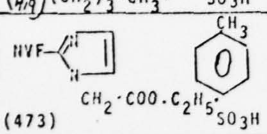
Compd.	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects 1 to 3 days after last dose: % inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
Panfuran S	S-463 S-464 S-484		IV, 53	500 mg/kg b.i.d. 7 doses + 250 mg/kg 1 dose	65	• 72	78	N.D. Hatch -	100	N.D. Surviving	
			IV, 54	250 mg/kg b.i.d. 8 doses	50	68	82	N.D. Hatch -	100	Mice used 1 day after last dose for short term effects	
			IV, 69	75 mg/kg b.i.d. 10 doses	0	47	40	89 Hatch +	35	64	29
HS-115 (Boehringer)	S-512 S-591 S-592		IV, 91	100 mg/kg b.i.d. 10 doses	90	83	89	98	70	N.D.	
			IV, 166	50 mg/kg b.i.d. 10 doses	60	37	30	15	0	28	0
			IV, 176	25 mg/kg b.i.d. 10 doses	0	N.D.	N.D.	N.D.	N.D.	14	0
HS-126 (Boehringer)	S-513		IV, 92	100 mg/kg b.i.d. 10 does	0	76	82	100	70	16	0
			IV 245	200mg/kg bid 10 doses	?5					97	83

Table II -*continued

SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
0789	H-34		IV, 273	250 mg/kg b.i.d. 10 doses	100						
			V, 179, 123	125 mg/kg b.i.d. 10 doses	0	98	96	80	100	100	100
0790	H-35	 (295)	IV, 272	125 mg/kg b.i.d. 10 doses	0	12	0	0	10	8	0
0858	H-36	 (206)	V, 102	250 mg/kg b.i.d. 6 doses, 25% Crem. El.	0	63	58	50	85	79	28
0360	H-37	 (304)	V, 125	200 mg/kg b.i.d. 5 doses, Crem. El.	100 (projected)	86	90	76	100		
				125 mg/kg b.i.d. 6 doses, 25% Crem. El.	10	52	47	67	60	78	50
0861	H-38	 (304)	V, 103	250 mg/kg b.i.d. 6 doses, Crem. El.	0	56	58	50	60	61	0
0864	H-39	 (Hg)	V, 107	250 mg/kg b.i.d. 6 doses, Crem. El.	0	41	36	7	0	49	0
0865	H-40	 (473)	V, 106	250 mg/kg b.i.d. 6 doses Crem. El.	0	0	0	15	0	46	0

*) H-34

b.i.d. 6 doses	% red.	% cures
100 mg/kg	100	100
50 mg/kg	68	17
25 mg/kg	43	0

Table II — continued

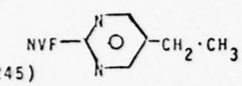
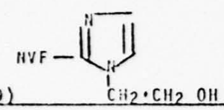
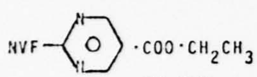
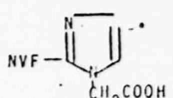
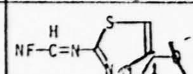
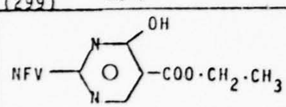
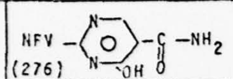
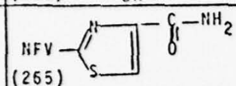
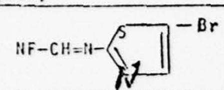
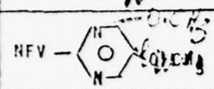
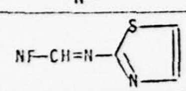
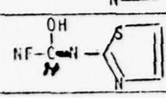
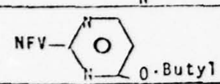
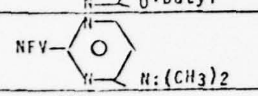
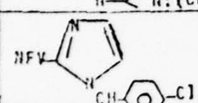
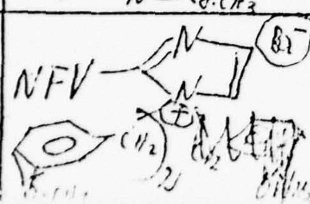
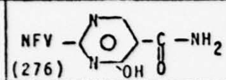
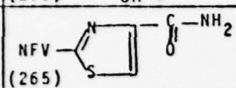
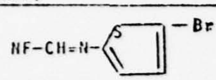
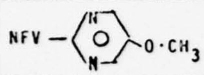
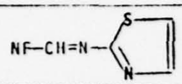
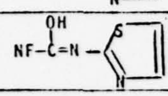
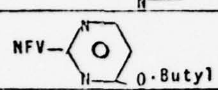
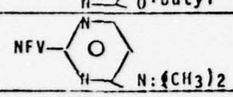
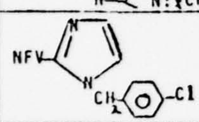
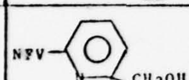
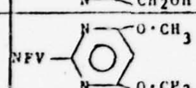
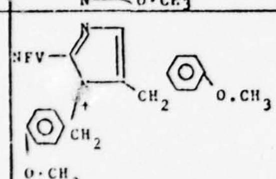
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parastiol. cures
0866	H-41	 (266)	V, 104	250 mg/kg b.i.d. 6 doses, Crem. El.	0	0	0	33	0	15	0
0867	H-42	 (245)	V, 105	200 mg/kg b.i.d. 6 doses, Crem. El.	0	82	84	71	85	40	0
0868	H-43	 (249)	V, 124	200 mg/kg b.i.d. 6 doses, Crem. El.	0	63	58	52	40	58	0
870	H-44	 MW:289	V, 122	250 mg/kg b.i.d. 6 doses, Crem. El.	0	0	0	15	0	0	0
869	H-45	 MW:249	V, 123	250 mg/kg b.i.d. 6 doses, Crem. El.	0	0	0	17	0	0	0
841	H-46	 (299)	V, 170	250 mg/kg b.i.d. 6 doses, Crem. El.	30	0	0	13	0	0	0
871	H-47	 (305)	V, 180	250 mg/kg b.i.d 6 doses, Crem. El.	0	0	0	13	0	0	0

Table II - continued

9 SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose) % reduction in # of worms	% parasitol. cures
872	H-48		V, 179	250 mg/kg b.i.d. 6 doses Crem. El.	10	0	0	12	0	0	0
873	H-49		V, 181	250 mg/kg b.i.d. 6 doses Crem. El.	0	24	28	.0	16	32	0
842	H-50		VI, 6	400 mg/kg b.i.d. 6 doses Crem. El.	0	8	12	10	0	8	0
874	H-51		V, 219	250 mg/kg b.i.d. 6 doses Crem. El.	0	88	96	80	50	83	25
844	H-52		V, 242	250 mg/kg b.i.d. 6 doses Crem. El.	0	8	6	3	0	0	0
843	H-53		VI, 7	250 mg/kg b.i.d. 6 doses Crem. El.	10	4	8	5	0	0	0
875	H-54		V, 243	250 mg/kg b.i.d. 6 doses Crem. El.	0	16	20	5	0	10	0
876	H-55		V, 211	250 mg/kg b.i.d. 6 doses Crem. El.	0	52	46	52	15	88	33
877	H-56		VI, 5	400 mg/kg b.i.d. 6 doses Crem. El.	0	55	47	62	10	86	0
880	H-57		VI, 137	250 mg/kg b.i.d. 6 doses; Crem. El	25	48	32	25	0	16	0
1100	H-58		VI, 137	500 mg/kg b.i.d. 6 doses, Crem. El	0	3	9	4	0	0	0

9

Table II - continued

SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	damage to reprod. system	Hepatic shift	Long term effect (4 to 5 weeks after last dose) reduction in # of worms	parasitol. cures
872	H-48		V, 179	250 mg/kg b.i.d. 6 doses Crem. El.	10	0	0	12	0	0	C
873	H-49		V, 181	250 mg/kg b.i.d. 6 doses Crem. El.	0	24	28	.0	16	32	0
842	H-50		VI, 6	400 mg/kg b.i.d. 6 doses Crem. El.	0	8	12	10	0	8	0
872	H-51		V, 219	250 mg/kg b.i.d. 6 doses Crem. El.	0	88	96	80	50	83	25
844	H-52		V, 242	250 mg/kg b.i.d. 6 doses Crem. El.	0	8	6	3	0	0	0
843	H-53		VI, 7	250 mg/kg b.i.d. 6 doses Crem. El.	10	4	8	5	0	0	0
875	H-54		V, 243	250 mg/kg b.i.d. 6 doses Crem. El.	0	16	20	5	0	10	0
876	H-55		V, 211	250 mg/kg b.i.d. 6 doses Crem. El.	0	52	46	52	15	88	33
877	H-56		VI, 5	400 mg/kg b.i.d. 6 doses Crem. El.	0	55	47	62	10	36	0
—	Furan-one		VI, 137	250 mg/kg b.i.d. 6 doses Crem. El.	0	33	39	61	0	16	0
880	H-57		VI, 137	250 mg/kg b.i.d. 6 doses Crem. El.	25	48	32	25	0	16	0
1100	H-58		VI, 137	500 mg/kg b.i.d. 6 doses Crem. El.	0	0	0	9	4	0	0

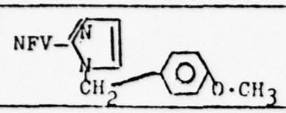
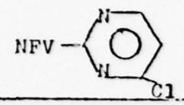
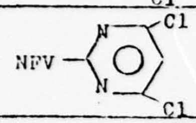
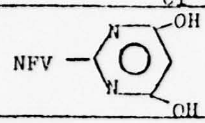
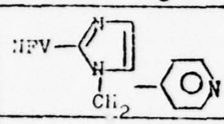
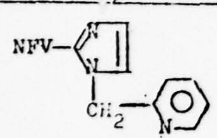
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose) % reduction in # of worms	% parasitol. cures
1101	H-59		VI, 137	375 mg/kg b.i.d. 6 doses Crem. El.	0	88	79	71	90	97	50
1102	H-60		VI, 155	280 mg/kg b.i.d. 6 doses Crem. El.	0	23	27	45	0	16	0
989	H-61		VI, 155	300 mg/kg b.i.d. 6 doses Crem. El.	0	8	0	20	0	0	0
881	H-62		VI, 155	280 mg/kg b.i.d. 6 doses	0	0	0	3	0	0	0
990	H-63	NF-CH=N-NH-C(=O)-CH_3									In Vitro
1054	H-64	NT-CH=N-N(H)-C(=O)-CH_3									
992	H-65		V, 174	350 mg/kg b.i.d. 6 doses	15	83	81	62	60	79	33
993	H-66		V,174	350 mg/kg b.i.d. 6 doses	15	30	58	25	20	0	0

Table II - continued

Table II - continued (header tokens "II")

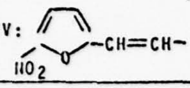
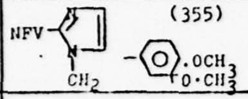
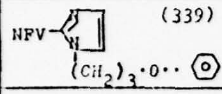
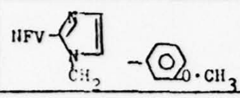
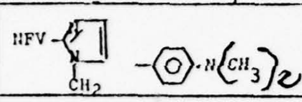
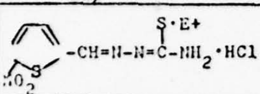
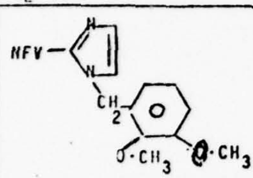
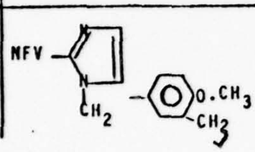
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% damage to reprod. system	% Hepatic shift	Long term effect (4 to 5 weeks after last dose) % reduction in # of worms	% parasitol. cures
		NFV: 									
0997	H-67	 (355)	VI, 181	400 mg/kg b.i.d. 6 doses Crem. El.	0	32	28	36	20	32	0
0994	H-68	 (339)	VI, 187	400 mg/kg b.i.d. 6 doses Crem. El.	0	39	34	42	0	40	0
0995	H-69		VI, 191	400 mg/kg b.i.d. 6 doses Crem. El.	0	72	85	83	60	95	50
0996	H-70		VI, 191	400 mg/kg b.i.d. 6 doses Crem. El.	0	58	69		50	20	0
1103	H-71		VI, 191	100 mg/kg b.i.d. 6 doses Crem. El.	0	15	12	45	0	0	0
998	H-72		VI, 198	100 mg/kg b.i.d. 6 doses Crem. El.	10	24	28	28	0	0	0
999	H-75		VI, 199	400 mg/kg b.i.d. 6 doses	0	22	19		0	50	0

Table II - continued

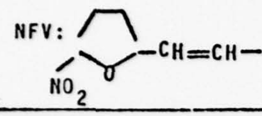
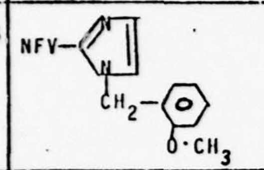
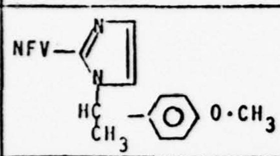
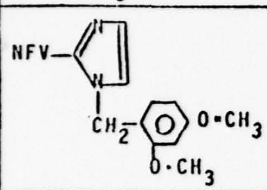
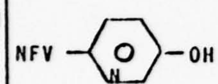
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose)			Long term effect (4 to 5 weeks after last dose)	
		NFV: 				% Inhibition of phosph. phosphatase	% reduction of glycogen levels	% Hepatic shift	% reduction in # of worms	% parasitol. cures
1105	H-76		VI, 202	400 mg/kg b.i.d. 6 doses	0	8	13	0	27	0
1106	H-77		VI, 202	200 mg/kg b.i.d. 6 doses	0	0	0	4	14	0
1107	H-78		VI, 207	400 mg/kg b.i.d. 6 doses	0	56	50		0	0
1000	H-80		VI, 210	350 mg/kg b.i.d. 6 doses	0	39	34	7	0	0
1109	H-81		VI, 210	250 mg/kg b.i.d. 6 doses	0	18	23	16	0	0

Table II - continued

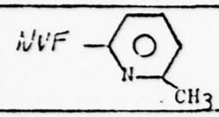
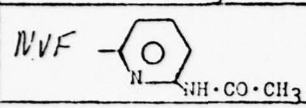
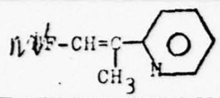
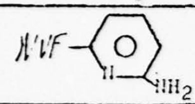
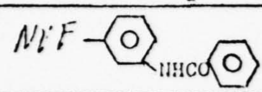
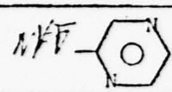
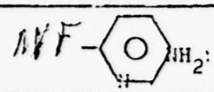
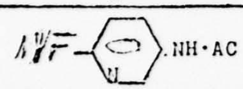
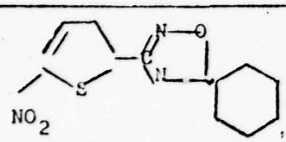
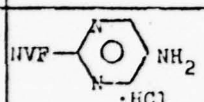
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% Hepatic shift	Long term effect (4 to 5 weeks after last dose) % reduction in # of worms	% parasitol. cures
1110	H-82		VI,232-33	250 mg/kg b.i.d. 6 doses	0	63	67	0	68	0
				400 mg/kg b.i.d. 6 doses	0				100	100
1111	H-83		VI,232-33	250 mg/kg b.i.d. 4 doses 125 mg/kg b.i.d. 2 doses	30	35	32	0	0	0
1113	H-84		VI,232-33	250 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1112	H-85		VI,232/33	250 mg/kg 2 doses 125 mg/kg 3 doses	65	33	36	10	21	0
1114	H-86		VI.232/33	350 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1115	H-87		VI,235	250 mg/kg b.i.d. 4 doses	60	48	59	0	62	0
1117	H-88		VI,235	250 mg/kg b.i.d. 6 doses	0	45	37	25	98	67
1116	H-89		VI,235	250 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1036	H-90		VIII,12	200 mg/kg b.i.d. 6 doses	0	21	30	50	90	14
				100 mg/kg b.i.d. 6 doses	0	7	6	0	48	0
1118	H-91		VIII,11	300 mg/kg b.i.d. 6 doses	0	89	88	75	100	100
				150 mg/kg b.i.d. 6 doses	0	81	82	45	42	0

Table II - continued

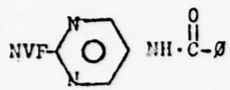
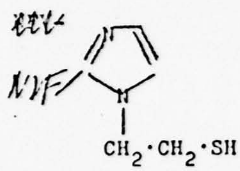
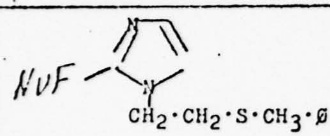
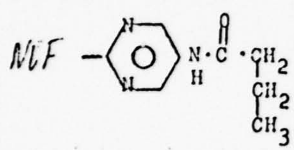
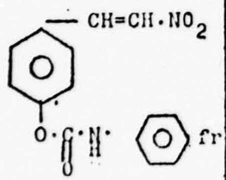
SB #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose): % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% Hepatic shift	Long term effect (4 to 5 weeks after last dose): % reduction in # of worms	% parasitol. cures
1120	H-92		VIII,17	400 mg/kg b.i.d. 6 doses	70	100	100	100	100	100
				200 mg/kg b.i.d. 6 doses	20	100	100	100	100	100
			VII,124	125 mg/kg b.i.d. 6 doses	10				100	100
			VII,146	100 mg/kg b.i.d. 6 doses	0				100	100
			VII,124	60 mg/kg b.i.d. 6 doses	0				78	50
1119	H-93		VIII,18	400 mg/kg b.i.d. 6 doses	0	63	56	65	67	0
				200 mg/kg b.i.d. 6 doses	0	41	36	50	42	0
1122 BE 17624	H-94 MP 208/210°		VII,60	400 mg/kg b.i.d. 6 doses Crem. El.	0	15	12	0	0	0
				250 mg/kg b.i.d. 6 doses Crem. El.	0	9	8	0	0	0
1121 BE 17615	H-95 MP 135		VII,60	400 mg/kg b.i.d. 6 doses	0	100	100	100	100	100
			VII,107	200 mg/kg b.i.d. 6 doses	0				0	0
				100 mg/kg b.i.d. 6 doses	0				0	0
1406 BE 55955	H-96		VII,146	200 mg/kg b.i.d. 6 doses	0	96	88	100	100	100
1428 BE 58269	H-97	 363	VII,147	300 mg/kg b.i.d. 6 doses	0	0	0	0	0	0

Table II - continued

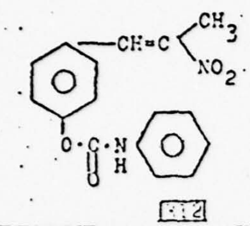
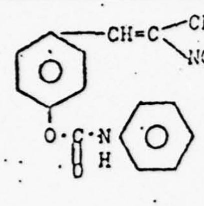
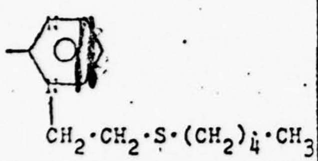
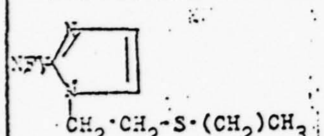
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effects (3 days after last dose) % Inhibition of phosph. phosphatase	% reduction of glycogen levels	% Hepatic shift	Long term effect (4 to 5 weeks after last dose % reduction in # of worms	% parasitol cures
1429	H-98 BE 58278		VII,147	300 mg/kg b.i.d. 6 doses	0	21	16	0	19	0
1430	H-99 BE 58287		VII,147	300 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1475	H-100		VII, 161	300 mg/kg b.i.d. 6 doses	0	73	62	30	79	50
1477	H-101 BE 01091		VII, 173 VII, 191	400 mg/kg b.i.d. 6 doses 200 mg/kg b.i.d. 6 doses	0 0	96 98	91 92	100 100	100 100	100 100
0490	H-19		IV, 243	150 mg/kg b.i.d. 6 doses	0				47	0
1481			VIII, 28	300 mg/kg b.i.d 6 doses	30	0	0	0	0	0

Table II - continued

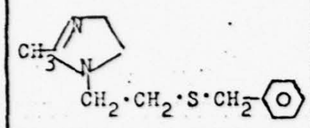
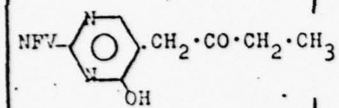
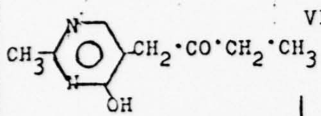
SR #	JHU #	Structure	Book + Page #	Dosage Schedule	Mouse Mortality %	Short term effect (3 days after last dose) % inhibition of phosph. phosphatase	% reduction of glycogen levels	% Hepatic shift	Long term effect (4 to 5 weeks after last dose) % Reduction in # of worms	% parasitol cures
1476 BG 01082	H-102		VII, 175	10 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1478 BG 11631	H-103		VII, 189	400 mg/kg b.i.d. 6 doses	0	8	0	0	0	0
1479 BG 11622	H-104		VII, 189	200 mg/kg b.i.d. 6 doses	0	0	0	0	0	0
1477	101	see page 15	VIII, 45	100 mg/kg, b.i.d. 6 doses	0	68	63	37	34	0
			VIII,45	50 mg/kg, b.i.d. 6 doses	0	18	16	0	0	0

Table II - continued

Since many nitroheterocyclic compounds have been reported to be mutagenic and carcinogenic (Cohen et al., J. Natl. Cancer Inst. 51, 403, 1973, Yahagi et al., Cancer Research 34, 2266, 1974) the mutagenic activities of those nitrovinylfuranes that had marked antischistosomal properties were determined, according to Ames et al., (Mut. Res. 31, 347, 1975), using *Salmonella typhimurium* strain TA98 and TA100 without and with an added rat liver microsomal fraction (Sq). All compounds proved mutagenic. Nitrovinylfuranes with an imidazole substituent exhibited the lowest activity, yet even within this group significant mutagenic effects were detected, with one *Salmonella* strain (TA100) while there was little or no mutagenic activity with another strain, TA98 (Table III).

TABLE III

Mutagenic activities of three antischistosomal nitrovinylfuranes with imidazole substituents

Compound	moles per plate	Number of revertants in of controls			
		TA-98		TA-100	
		without SQ	with SQ	without SQ	with SQ
	5	1	0	12(2.4)	(1.4)
H-36(SQ0858)*	15	3	2	38(2.55)	23(1.7)
	50	5	0	114(2.3)	80(1.6)
	5	2	0	16(3.2)	13(2.6)
H-59(SQ1101)*	15	5	3	50(3.3)	42(2.7)
	50	11	5	152(3.0)	117(2.3)
	0.05	0	3	8(160)	57(1,140)
H-101(SQ1477)*	0.15	2	7	23(146)	156(1,040)
	0.50	0	12	74(148)	550(1,100)

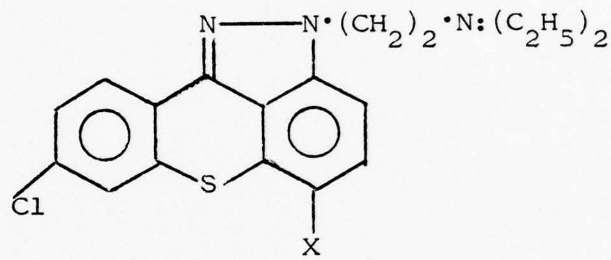
*See Table II for structural and biological properties of this compound.

3. Dissociation of antischistosomal from mutagenic activity of hycanthone analogs

As reported previously, two chloroindazole analogs of hycanthone, a widely used antischistosomal drug, have been shown to have little or no mutagenic activity. In contrast to hycanthone, these compounds failed to exhibit hepatoxic and malignant cell-transforming activities. Their acute toxicity is 5 to 10 times lower than that of hycanthone. Further studies of the antischistosomal activity of these two compounds showed that they are more active than hycanthone when administered as a single oral dose to mice infected with Schistosoma mansoni. (Table IV)

TABLE IV

Antischistosomal activities of two chloroindazole thioxanthenes administered as a single oral dose (gastric intubation) to mice infected with Schistosoma mansoni



X	Single oral dose mg/kg	Number of mice	% reduction in number of worms	% parasitol. cures	% of mice without hatching eggs
CH_3	160	11	97	64	82
	80	13	67	0	69
CH_2OH	160	14	100	100	100
	80	12	98	82	100
Hycanthone	160	11	83	0	27
	80	12	66	0	9

Since the N-oxide derivative of hycanthone had 10 times lower mutagenic activity than hycanthone itself, the N-oxide derivatives of the two chloroindazole derivatives have been prepared.

N-oxidation of the distal amino group resulted in a marked reduction in mutagenicity. In the case of the N-oxide of IA-4 both mutagenicity and acute toxicity was reduced significantly, while antischistosomal activity was unchanged. Accordingly, the chemotherapeutic index of IA-4 N-oxide is more than 12 times higher than that of hycanthone (Table V).

TABLE V

Comparative antischistosomal and toxicological properties of two chloroindazole hycanthone analogs and of their N-oxide derivatives

Compound	ED_{50} *) mg/kg	LD_{50} **) mg/kg	Comparative mutagenic activity ***)	$\frac{LD_{50}}{ED_{50}}$ ****)
Hycanthone	30	250	100	8.3
IA-3	60	2,500	5.2	41.5
IA-3 N-oxide	15	700	0.4	47
IA-4	30	1,250	7.4	42
IA-4 N-oxide	30	3,500	1.5	117

*) Single i.m. dose producing 50% reduction in the number of worms in mice infected with Schistosoma mansoni.
**) Median single i.m. lethal dose for mice.
***) % of mutant colonies of a Salmonella strain TA 1538 induced by the same concentration of hycanthone (0.025 uM/ml.)
****) Chemotherapeutic index.

As in the case of IA-4, IA-4 N-oxide is equally active when administered orally or intramuscularly. A well-tolerated oral preparation of an anti-schistosomal drug would be preferable to a parenterally administered formulation not only because of its reduced cost but also because of toxicological considerations. Analysis of the muscular site in mice that had been injected with hycanthone revealed the presence of a number of products that persisted for at lease 8 months in this tissue. The quantity of these materials gradually decreased over this time period, indicating either a slow destruction or release into the general circulation. Several separate components of this depot are recognizable by thin-layer chromatography of the extracted depot. None of the components was identical with hycanthone; one has been identified as the dimer of hycanthone; and the mutagenic activity of another was 3 times as high as that of hycanthone. Slow release of such a component might be related to the hepatocarcinogenic effect of hycanthone in mice infected with Schistosoma mansoni (Haese et al., J. Pharm. Exp. Ther., 186, 430, 1973).

Thioxanthenes have been considered to have no antischistosomal prophylactic activities. Therefore, it is noteworthy that high doses of IA-4 N-oxide are effective in eliminating the development of the infection when administered one hour prior to, or one day after, exposure of mice to cercariae (Table VI).

TABLE VI

Prophylactic effects of single dose of IA-4 N-oxide in mice exposed to 100 cercariae 1 hour after or one day before treatment.

The number of adult worms are determined by autopsy of the mice 60 days after infection with cercariae.

Time of treatment	Number of treated mice	Dose mg/kg	Route	% reduction in number of worms	% of mice without worms
1 hr prior to exposure to cercariae	14	400	i.m.	100	100
	12	400	oral	100	100
	11	200	i.m.	100	100
	14	150	i.m.	96	85
	15	150	oral	98	87
	9	80	i.m.	73	11
24 hrs after exposure to cercariae	12	400	i.m.	100	100
	16	200	i.m.	99	88
	15	200	oral	100	100
	16	150	i.m.	94	75
	17	150	i.m.	90	71
	12	80	i.m.	52	50

4. Metabolism of IA-4 and of IA-4 N-oxide

An effort has been made to trace the metabolic fats of two hycanthone analogs, the chloroindazoles IA-4 and IA-4 N-oxide.

A system was developed for solvent extraction of IA-4 and of its metabolites from tissues. The method consists of homogenizing the tissue in four volumes of borato buffer (pH 9.4) in a glass homogenizer, adding 10 volumes of a solvent mixture (25% cidhloromethane and 75% butyl chloride) and mix with shaking for 30 minutes. After low speed centrifugation (500 rpm for 5 minutes) the organic phase is removed with a pipette. When the phases don't separate well, re-extraction will improve the yield of the solvent phase.

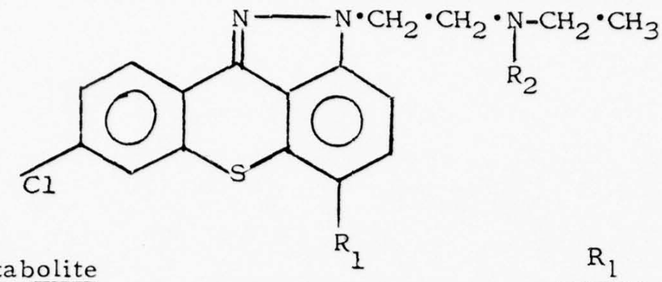
When IA-4 is added prior to homogenization it can be recovered quantitatively.

If it is necessary to remove lipid contaminants, the aqueous homogenate can be extracted with hexane prior to the chlorinated hydrocarbon extraction. Apparently, hexane was not to remove any drug-related material.

After administration of IA-4 to mice the livers of these animals contain large amounts of three metabolites and smaller amounts of at least two others. High pressure liquid chromatography using a pellicular silica column (2.7mm X 50 cm packed with Vydac approx. 30u) and a mixture of methanol and dichloromethane (1.5% to 10.5% methanol in a linear gradient for 15 ml at 2 ml/min) as eluent has proved quite efficient for separation and analysis of this mixture. The extract is reduced in volume by evaporation under N_2 and the concentrated extract is injected on the column 5 ul to 25 ul at a time. Since these metabolites degrade readily to very polar products on exposure to air it is important not to dry the sample completely. In fact, any concentrated extract must be diluted for storage. Samples stored even in low concentrations at $4^{\circ}C$ or at $-35^{\circ}C$ are not stable for more than a few days.

The chromatography of extracts of tissues and worms from IA-4 treated mice readily reveals the drug and three metabolites. These have been isolated and mass spectra consistent with the following structures were obtained.

Metabolites of IA-4 and of IA-4 N-oxide in mice



Metabolite		R_1	R_2
A	Desethyl IA-4	CH_2OH	H
B	Desethyl IA-4 aldehyde	CHO	H
C	IA-4 aldehyde	CHO	$CH_2.CH_3$

Two and a half hours after oral administration of 375 mg/kg of IA-4 all metabolites in the liver reach this highest level. Thereafter, the levels gradually decline with small amounts remaining even after 20 hours. The level of the parent compound is maximal after about 4 hours. At maximal level, metabolite A reaches about 120 ug/g of tissue (wet weight), metabolite B 100 ug/g and C is present at about 20 ug/g. The IA-4 itself reaches a level of about 40 ug/g. Other tissues, in particular peripheral blood, heart kidney and spleen, yield measurable amounts of these metabolites. The relative amounts of each metabolite and the time course of the levels in these tissues were similar to those in the liver while the actual concentrations are approximately an order of magnitude lower.

Although the levels of metabolites, A, B and C are very low (in the order of 2 ug/g) in mouse blood, this low level decays slowly. (Half life 20 hours) Only traces are found in the serum fraction, the cells containing nearly the entire amount present in blood. It would thus appear that IA-4 and its metabolites are taken up by the worms as a consequence of their ingestion of red blood cells.

Using a slightly different extraction techniques, liver tissue yielded another component which is characterized by relatively high polarity. This component P fails to migrate on TLC developed with 75% methanol/25% dichloromethane. Component P contains at least 2 different metabolites which so far have not been resolved.

The antischistosomal effectiveness of IA-4 N-oxide is equal to that of IA-4, while it has much lower metagenic activity than IA-4. When the same dose of IA-4 N-oxide (375 mg/kg) was administered to mice, the same metabolites A, B. and C in the same absolute as well as relative amounts were found in the liver and the level versus time course was similar. However, neither IA-4 N-oxide nor IA-4 itself could be detected. Another difference was a much reduced level of component P when IA-4 N-oxide was administered. The lower mutagenic activity of IA-4 N-oxide suggests that component P may be responsible for the mutagenic activity.

Studies of the metabolic products of IA-4 were extended to their determination in both male and female schistosomes following oral administration of IA-4 to the host. While maximal levels in host tissues were reached after 2-1/2 hours, the levels of the drug and the three metabolites were still rising in the worms after 16 hours. Maximal levels were found about 42 hours after oral administration of IA-4. These levels reached levels which, on a per gram basis, were much higher than any observed in the host. For example, the diethyl IA-4 aldehyde in female worms averaged 430 ug/g. High levels remained even after 96 hours.

On the other hand, the <u>relative</u> amounts of the drug and its metabolites were similar in host and parasite tissues, both diethyl compounds being 10 to 20 times more abundant than the drug or its aldehyde. When IA-4 N-oxide was administered to the host, no trace of IA-4 or the N-oxide could be found in the worms although metabolites A, B and C were present at levels similar to those found after the same dose IA-4. Since the parent compounds have little effect on the worms *in vitro*, one of these compounds might be the active antischistosomal metabolite.

There were no significant differences in the concentrations of IA-4 or of its metabolites in hycanthone-susceptible and hycanthone-resistant schistosomes. Since the latter are completely cross-resistant to IA-4, this type of drug resistance cannot be ascribed to difference in the uptake of the drug and of its metabolites between hycanthone-susceptible and hycanthone-resistant schistosomes.

5. Mutagenic activities in vitro and in vivo of 5 antischistosomal compounds

Five antischistosomal compounds - hycanthone, two of its chloroindazole analogs (IA-4 and IA-4 N-oxide), oxamniquine, and metrifonate - were tested for mutagenic activity, using *Salmonella typhimurium* strains TA 98 and TA 100 under *in vitro* and *in vivo* (host-mediated) conditions. In all assay systems hycanthone exhibited for far the highest mutagenic potency. Although oxamniquine and metrifonate had low mutagenic activity *in vitro*, and although their administration resulted in urine of low mutagenic activity, their host-mediated mutagenic activities on strain TA 100 were fairly high. Confirming earlier studies with a less sensitive *Salmonella* strain, TA 1535 (Hartman *et al.*, 1975, Hulbert *et al.*, 1974), IA-4 N-oxide was found to be less mutagenic than IA-4. Orally administered IA-4 and IA-4 N-oxide were less mutagenic under *in vivo* conditions than an equal dose administered intramuscularly. By contrast, the antischistosomal activity of a given dose of each compound was the same, regardless of which of these two routes was used, suggesting that mutagenic and antischistosomal effects are produced by different metabolites. The observations reported in this report provide additional evidence that mutagenic activities can be dissociated from desired chemotherapeutic effects by suitable structural modifications.

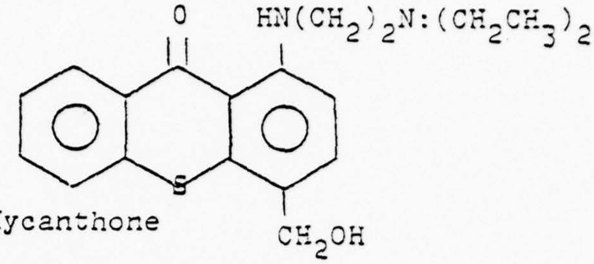
McCann *et al.*, (1975) and McCann and Ames (1976) have shown that most known carcinogenic compounds exhibit mutagenic effects on certain strains of *S. typhimurium*. Conversely, few compounds found to be non-carcinogenic exhibited mutagenic activity. Because of this close correlation the results of mutagenic assays provide a high degree of probability in predicting carcinogenic activity. While bacteria lack many enzymes catalyzing the metabolism of drugs in mammals, carcinogenic metabolites can be detected (a) by adding liver microsomes *in vitro* to the bacteria incubated with the test substance (Ames *et al.*, 1973, 1975; Malling, 1971), (b) by the incubation of bacteria in the peritoneal cavity of animals to which the test substance has been administered (Legator and Malling, 1971), or (c) by determination of the mutagenic activity of the urine of these animals (Gabridge *et al.*, 1969; Commoner *et al.*, 1974; Durston and Ames, 1974).

Approximately 200 million human subjects are infected with schistosomiasis. With a population of this magnitude, the need for an evaluation of the mutagenic and carcinogenic potential of available antischistosomal drugs is evident. During the past 6 years over one million human subjects infected with *Schistosoma mansoni* and *Schistosoma hematobium* have been treated with the thioxanthone derivative hycanthone. This drug is a potent mutagen in submammalian and mammalian cells (Clive, 1974; Clive *et al.*, 1972; Green *et al.*, 1973a; Green *et al.*, 1973b; Hartman *et al.*, 1973; Hartman *et al.*, 1975; Hartman *et al.*, 1971; Knaap and Kramers, 1973; Lee, 1973; von Borstel and Quah, 1973; for a review, see Hartman and

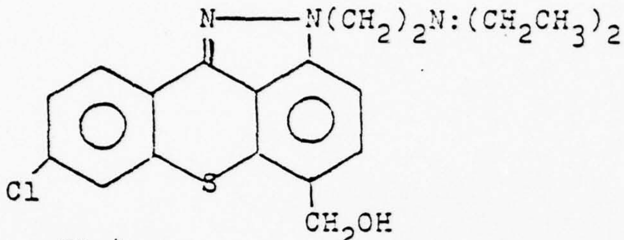
Hulbert, 1975) and has been recommended as a reference compound for a variety of mutagenic assay systems (Drake, 1975). In addition, hycanthone has been reported to be teratogenic (Moore, 1972), to induce malignant transformations (Hetrick and Kos, 1973), breaks in double-stranded DNA (Sarma et al., 1976), and hepatocarcinomas in mice infected with Schistosoma mansoni (Haese et al., 1973; Haese and Bueding, 1976). While there is some evidence that in a variety of systems mutagenic activities of other antischistosomal compounds are lower than those of hycanthone (Clive, 1974; Hartman et al., 1973; Hartman et al., 1975; Hulbert et al., 1974; Kramers and Knaap, 1975; Meadows et al., 1973; Ray et al., 1975; von Borstel and Quah, 1973), a more detailed comparison using two sensitive tester strains, under both in vitro and in vivo conditions, would provide an evaluation of the mutagenic and carcinogenic potential of each of these compounds. Using S. typhimurium strains TA 98 and TA 100 developed by Ames et al., (1975) the mutagenic activities of five antischistosomal drugs, hycanthone, oxamniquine, metrifonate, IA-4 and IA-4 N-oxide (structures in Fig. 1), under in vitro and in vivo (host-mediated) conditions, have been determined. During the past 8 years the effectiveness of the organic phosphorus compound metrifonate in the treatment of infections caused by Schistosoma hematobium has been reported (Davis and Bailey, 1969; Forsyth and Rashid, 1967; Gentilini et al., 1973; Hanna et al., 1966; Niemandt and Murahwa, 1975; Reddy et al., 1975). More recently, a number of clinical investigations have revealed that orally administered oxamniquine is as effective as intramuscularly injected hycanthone in infections produced by Schistosoma mansoni (Blair and Weber, 1975; da Silva et al., 1974; da Silva et al., 1975; Katz et al., 1976; Koura et al., 1975; Ongom et al., 1975; Siongok et al., 1975). The antischistosomal activities in mice of IA-4 and IA-4 N-oxide, two chloroindazole analogs of hycanthone, are similar to that of the latter (Blair and Weber, 1975; Bueding, 1975; Hulbert et al., 1974), but their mutagenic and other toxic properties are considerably lower (Bueding, 1975; Bueding et al., 1973; Clive, 1974; Haese et al., 1973; Haese and Bueding, 1976; Hartman et al., 1973; Hartman and Hulbert, 1975; Hartman et al., 1975; Hetrick and Kos, 1973; Hulbert et al., 1974; Kramers and Knaap, 1975; Lucier et al., 1973; Meadows et al., 1973; Sauro and Green, 1973; von Borstel and Quah, 1973).

S. typhimurium cultures of TA100 and TA 98 (McCann et al., 1975b) were obtained from Dr. Bruce Ames and from Dr. Philip E. Hartman. These cultures were reisolated fortnightly on nutrient agar plates (Difco) containing 80 ugm ampicillin. The isolates were checked for sensitivity to UV light, toxic effects of crystal violet and mutagenic effects of hycanthone, and were stored in tightly capped half-dram vials at -80°C in 9% DMSO nutrient broth. Multiple samples were made of each culture and each vial was discarded after a single use. All assays were performed with an overnight culture of reisolated strains grown in double strength nutrient broth (16 gm Difco Nutrient Broth and 3 gm NaCl per liter). Cultures were kept

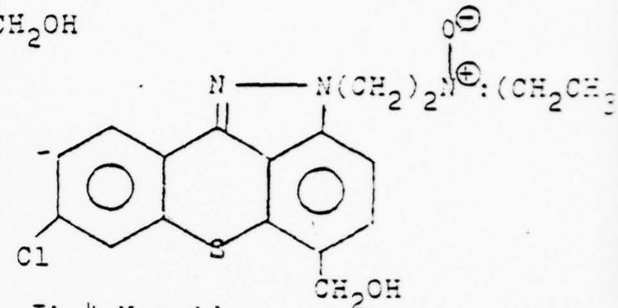
Figure 1



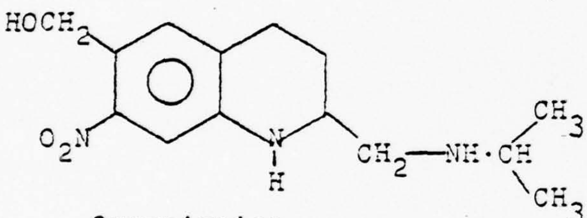
Hycanthone



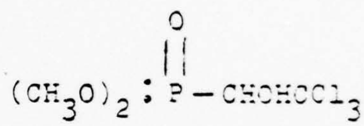
IA-4



IA-4 N-oxide



Oxamniquine



Metrifonate

at 4^{o}C until used. All assays were run with standards to check background nutation rates, bacterial population size and sensitivity to mutations in the presence of hycanthone (ug per ml). All assays were run on Vogel-Bonner E agar medium (Vogel and Bonner, 1956) supplemented with 2.5% nutrient broth, 1% glucose and 2mM biotin.

In vitro assays

The growth inhibitory activity of each drug was determined by growing Salmonella TA100 on agar plates in the presence of added 6 mM histidine with varying concentrations of the compound. The percentage survival was determined after incubation at 37^{o}C for 48 hours. In the subsequent in vitro mutagenic assays, the maximal concentration used did not exceed the one producing 10% growth inhibition. Three, ten, and, whenever indicated, thirty times lower concentrations were used also.

Mutagenic assays were carried out with and without added S9 (microsomal) fractions of phenobarbital treated rats as described by Ames et al., (1973, 1975). With the compounds used in this study metabolic activation with phenobarbital induction was either equal to, or greater than, that observed with Aroclor-induced microsomes. All drugs were dissolved initially in DMSO. The final DMSO concentration of control and drug-containing plates was adjusted to 0.1 ml per 15 ml of medium. Plates were counted after 48 hrs.

Host-mediated assays

Host-mediated assays were performed by methods similar to those described by Legator and Malling (1971). Overnight bacterial cultures were diluted 4-fold with 2X nutrient broth and were reincubated at 37^{o}C for 2 hours. Two hours prior to administration of the bacteria, drugs were administered, either by gastric intubation or intramuscularly. Five 6- to 8-week-old female mice (mixed Swiss-Webster, CF-1, Charles River) were used for each dose level. 2 ml of the culture was injected intraperitoneally. After 6 hrs the bacteria were retrieved from the peritoneal cavity by the injection and removal of 2 ml of 0.85% NaCl. These exudates were plated to determine the number of revertants and a 1:10 (Clive, 1974) serial dilution was plated with 0.2 ml added nutrient broth to determine the number of viable bacteria. Plates were counted after 48 hrs of incubation at 37^{o}C and results were expressed in terms of revertants per million bacteria.

Urine analysis

Five female mice were placed into a metabolism cage and control urines were collected on ice for 24 hours. These animals were then treated with a given drug and urine was collected in refrigerated containers for the two subsequent 24-hour periods. The volume of the urine was measured and the urine was stored at -80°C until assayed.

Urine specimens were thawed, centrifuged at 10,000 X g for 15 min. and sterilized by filtration through a .22 um Millipore filter. Effects of 0.1 and 0.25 ml aliquot were tested on strains TA98 and TA100 with and without added S9 microsomal fraction. Plates were counted after incubation at 37°C for 48 hours. The dose response at these two urine concentrations was used to calculate the number of revertants induced by the total volume of urine excreted in 24 hours. In order to determine whether some mutagens were present as inactive urinary glucuronate or sulfate conjugates, aliquots of the mouse urines were incubated for 3 hrs at 37°C and pH 4 with beta-glucuronidase (12.5 m U per ml urine) - arylsulfatase (6.25 m U per ml urine) (Boehringer). Aliquots of this mixture were tested after adjustment to pH 7 in the absence and the presence of the S9 fraction.

Antischistosomal activities

Antischistosomal activities of hycanthone and of its two chloro-indazole analogs (IA-4 and IA-4 N-oxide) were determined and expressed as previously described (Bueding *et al.*, 1973).

Antischistosomal compounds

Hycanthone was kindly supplied by Dr. S. Archer, metrifonate by Dr. R. Gonnert, oxamniquine by Dr. R. Foster, and IA-4 by Drs. E. Elslager (Elsager, 1970) and F. de Serres. IA-4 N-oxide was prepared as reported previously (Hulbert *et al.*, 1974).

In vitro mutagenic assays

In agreement with observations of Sugimura[1]) all five compounds had mutagenic activity *in vitro.* There was a linear dose response within the range of drug concentrations tested. The mutagenic response of strain TA98 to IA-4 N-oxide represented the greatest deviation from linearity. Metrifonate had the lowest, and hycanthone had by far the highest mutagenic activity (Table VII). Rat liver microsomes enhanced the mutagenic activity of IA-4 and IA-4 N-oxide on strain TA100, but had little or no effect in altering the mutagenic effects of the other drugs on either of the two strains of *S. typhimurium.* In agreement with earlier studies using a less sensitive strain, TA1538 (Hartman *et al.*, 1975; Hulbert *et al.*, 1974), it was found that the mutagenic activity of IA-4 N-oxide was significantly lower than that of IA-4. Oxamniquine was 2 times more mutagenic than IA-4 N-oxide when strain TA100 was used but was equally mutagenic on strain TA98 (Table X).

TABLE VII

Mutagenic activities of 5 antischistosomal compounds on *S. typhimurium* strains TA-100 and TA-98 *in vitro* with and without added rat liver microsomal fraction (S9).

Numbers in parentheses indicate numbers of revertants per nmole.

Compound	n moles per plate	Number of revertants in excess of controls#			
		TA-100		TA-98	
		without S9	with S9	without S9	with S9
Hycanthone	25	396(15.8)	399(16.0)	341(13.6)	309(12.4)
	50	817(16.3)	815(16.3)	668(13.5)	625(12.5)
	100	1,609(16.1)	1,611(16.1)	1,371(13.7)	1237(12.4)
Oxam-niquine	364	51(.140)	61(.169)	65(.178)	48(.131)
	1,092	162(.148)	178(.163)	201(.184)	151(.138)
	3,640	575(.118)	464(.158)	612(.168)	441(.121)
Metrif-onate	3400	49(.014)	37(.011)	62(.018)	65(.019)
	10,200	153(.015)	142(.014)	189(.019)	182(.018)
	34,000	368(.011)	349(.010)	578(.017)	539(.016)
IA-4	155	24(.153)	43(.276)	40(.258)	42(.270)
	465	72(.155)	128(.284)	124(.264)	137(.294)
	1,550	209(.135)	398(.258)	394(.254)	410(.265)
IA-4 N-oxide	164	7(.043)	15(.091)	23(.140)	25(.150)
	492	18(.037)	46(.093)	63(.128)	65(.132)
	1,640	58(.035)	158(.098)	184(.112)	207(.125)

Spontaneous mutation background averaged 172 and 23 revertants per plate for TA-100 and TA-98 respectively.

TABLE VIII

I.P. Host Mediated Mutagenic Activities of 5 Antischistosomal Compounds

Activities are expressed in number of revertants per 10^6 bacteria in excess of controls. Standard errors of the mean are given in parentheses. Control levels were 0.68 ($\pm$.05) and 0.10 ($\pm$.03) for TA100 and TA98 respectively.

Compound	Route of Admin	Dose mg/kg	TA100	TA98
Hycanthone	i.m.	100	4.52($\pm$.30)	7.37($\pm$.39)
	i.m.	20	1.33($\pm$.12)	1.34($\pm$.18)
Oxamniquine	oral	200	.83($\pm$.06)	.10($\pm$.03)##
	oral	100	.43($\pm$.07)	NS#
	oral	50	.23($\pm$.04)	NS#
Metrifonate	oral	200	1.42($\pm$.11)	NS#
	oral	100	.79($\pm$.09)	NS#
	oral	50	.29($\pm$.06)	NS#
IA-4	i.m.	100	1.06($\pm$.12)	1.37($\pm$.11)
	oral	400	.62($\pm$.06)	1.37($\pm$.17)
	oral	100	.29($\pm$.07)	.20($\pm$.06)
IA-4 N-oxide	i.m.	100	.76($\pm$.03)	.32($\pm$.07)
	oral	400	.10($\pm$.02)###	.32($\pm$.09)
	oral	100	NS#	NS#

\# No significant difference from controls $p > .1$

\## $p = .05$

\### $p = .02$

TABLE IX

Mutagenic activities of the urines of mice treated with 5 antischistosomal compounds in the presence and absence of a rat liver microsome fraction (S9)

Figures indicate number of revertants in excess of controls per total daily urine excreted by one mouse

Compound	Route of Admin	Dose mg/kg	TA 100				TA 98			
			Day 1		Day 2		Day 1		Day 2	
			without S9	with S9	without S9	with S9	without S9	with S9	without S9	with S9
Hycan-thone	i.m.	100	868	650	550	205	625	3500	140	4600
	i.m.	20	268	221	183	86	229	1640	38	1260
Oxam-niquine	oral	200	33	10	27	0	14	8	4	2
	oral	100	25	12	0	0	7	3	0	0
Metrif-onate	oral	200	72	48	12	13	7	8	3	5
	oral	100	41	37	5	7	0	0	0	0
IA-4	i.m.	100	210	89	69	61	113	16	23	11
	oral	400	320	182	68	68	246	29	100	6
	oral	100	120	72	70	45	79	14	11	5
IA-4 N-oxide	i.m.	100	105	98	39	42	68	0	20	0
	oral	400	168	166	68	60	130	0	80	0
	oral	100	79	62	37	0	29	6	9	0

TABLE X

Relative mutagenic activities of 5 antischistosomal drugs in vitro and in vivo

Compound	Per cent of mutagenic activity of hycanthone					
	In vitro[1]		Host mediated[2]		Urine from drug treated mice[1,2,3]	
	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98
Hycanthone	100	100	100	100	100	100
Oxamniquine	1.01	1.3	9.5	0	1.7	.08
Metrifonate	.08	.13	17.5	0	3.2	0
IA-4	1.69	2.03	6.4	2.7	13.4	1.11
IA-4 N-oxide	.58	.99	0	0	8.1	.47

1) If the addition of rat liver microsomes resulted in an increase in the number of revertants, the latter figure was used in this tabulation.

2) The values are based on the mutagenic activity resulting from administration to mice of a therapeutic dose of 100 mg/kg. Hycanthone was administered I.M.; the other compounds, orally.

3) Based on the number of revertants in excess of controls induced by the total volume of urine excreted per mouse during 48 hours following drug administration.

TABLE XI

Antischistosomal activities and acute toxicities of hycanthone and of two of its chloroindazole analogs in mice infected with a Puerto Rican strain of *Schistosoma mansoni*

Figures indicate per cent reduction in the number of worms; figures in parentheses indicate percentage of mice with parasitological cures.

	Route	Hycanthone	IA-4	IA-4 N-oxide
LD_{50} (mg/kg)	i.m.	253	1,250	3,500
Dose (mg/kg)				
80	i.m.	92(22)	89(20)	94(32)
80	oral	66(0)	94(54)	98(82)
60	i.m.	88(14)	84(16)	86(21)
60	oral	55(0)	91(50)	91(55)

Host-mediated assay (Table VIII).

Again, hycanthone was by far the most potent mutagenic compound. Oxamniquine and metrifonate were considerably more mutagenic with TA100 in this assay than in vitro; with TA98 little or no mutagenic activity was observed. By contrast, IA-4 N-oxide and, to a lesser degree, IA-4 had much lower activity in vivo than in vitro. In fact, when a therapeutically effective oral dose of IA-4 N-oxide (100 mg/kg) was used, no mutagenic activity was detectable on either strain. It is of interest that IA-4 and IA-4 N-oxide administered intramuscularly are 4 times as mutagenic as the same dose given orally.

Mutagenic activities of urine from animals treated with antischistosomal drugs

As was the case in the other assays, hycanthone administration produced urine with the highest mutagenic activity (Table IX). Furthermore, highly significant increases were noted when urine from hycanthone-treated mice was tested with TA98 in the presence of microsomes. Administration of oxamniquine and metrifonate gave rise to urine containing less mutagenic activity than the specimens following the administration of IA-4 N-oxide. Treatment of the urines with beta-glucuronidase and arylsulfatase produced no increase in mutagenic activities over and above those observed with the microsomal (S9) fraction. Consistent with the in vitro and host-mediated assays, administration of IA-4 N-oxide gave rise to a urine of lower mutagenic activity than that of IA-4. As seen in the host-mediated assay, intramuscular administration of IA-4 and its N-oxide analog resulted in greater mutagenic activity than administration by the oral route.

While hycanthone proved to be by far the most potent mutagen in all three types of assays, the relative mutagenic activities of the other four compounds varied considerably depending on the nature of the assay (Table X). In vitro, metrifonate is the least mutagenic, while, in the host-mediated assay, IA-4 N-oxide exhibits the lowest mutagenicity; in fact, with this compound mutagenic activity was not detectable with an oral dose whose schistosomicidal activity is of a high order. Oxamniquine gave rise to urinary products of lower mutagenic potency than that of any of the other schistosomicides tested. Hence, a complete evaluation of the mutagenic potential of a given compound requires the use of several assay procedures.

Comparative antischistosomal activities of hycanthone and two of its chloroindazole analogs

Results recorded in Table XI indicate that in mice hycathone, IA-4 and IA-4 N-oxide are equipotent as schistosomicides when administered intramuscularly. However, when the same unit dose is administered orally, hycanthone is less effective, while both chloroindazole analogs maintain their activity. It is noteworthy that the acute toxicity (single intramuscular dose) of hycanthone is five or more than 12 times higher than that of IA-4 and IA-4 N-oxide, respectively.

In this study we have compared the mutagenic activities of five antischistosomal compounds in vitro as well as under two in vivo conditions. Confirming earlier studies (Hartman et al., 1975; Hulbert et al., 1974), it was found that N-oxidation of IA-4 reduces mutagenic activity both in vitro and in vivo, while antischistosomal activity was maintained. The intramuscular administration of either IA-4 or IA-4 N-oxide revealed four times higher mutagenic activity than the oral administration of the same dose. Yet, the antischistosomal potencies of these two compounds were equal when administered at the same dose by either of these two routes. This provides additional evidence that mutagenic and schistosomicidal effects can be dissociated from each other.

While with Salmonella strains TA100 and TA98 strongly positive mutagenic effects were observed in the host-mediated assay following the administration of hycanthone, Ray et al., (1975) reported negative results using the same in vivo assay, but a different tester strain, TA1534. Such results could be ascribed either to a much lower sensitivity of this strain, or to too short an exposure of the host to the drug (2 1/2 instead of 6 hours) or to an inadequate volume of fluid employed to retrieve the bacteria from the peritoneal cavity.

On a quantitative basis, there is a positive correlation between mutagenic and carcinogenic potencies among the five antischistosomal compounds tested in this study. The high mutagenic activity of hycanthone is paralleled by a significant hepatocarcinogenic effect of this compound following the administration of only a single dose of hycanthone to mice infected with S. mansoni (Haese et al., 1973). Only the most potent carcinogens induce neoplasms after a single dose (Weisburger and Weisburger, 1967). By contrast, a 3 time higher doses of oxamniquine and of IA-4 - whose mutagenic activities in vitro and in vivo are considerably lower - failed to induce hepatocarcinomas under these conditions (Haese and Bueding, 1976). Similar positive quantitative correlations are provided by the lower mutagenic potencies of IA-4 on yeast cells (Meadows et al., 1973; von Borstel and Quah, 1973), Drosophila (Knaap and Kramers, 1973; Kramers and Knaap, 1975), and on mammalian cell systems, both in vitro (Clive, 1974; Clive et al., 1972) and in vivo (Green et al., 1973a and 1973b; Sauro and Green, 1973). Also, in contrast to hycanthone, IA-4 failed to induce either malignant transformations of mammalian cells in vitro (Hetrick and Kos, 1973) or to cause breaks in double-stranded hepatic DNA in vivo (Sarma et al., 1976). Therefore, within this series of compounds, mutagenic potencies in Salmonella paralleled mutagenic potencies in other cell systems, including mammalian cells, as well as carcinogenic potencies. Within the limitations discussed by McCann and Ames (1976), these authors have reported other parallelisms between mutagenic and carcinogenic potencies, especially among structurally related compounds. Such correlations are by no means complete because they do not necessarily apply to all chemicals. However, some discrepancies might be more apparent than real. In fact, they might diminish or even disappear with the development of improved methodologies of the mutagenic assay systems. Whether or not, aside from hycanthone, the other four antischistosomal compounds are, albeit weaker, carcinogens, could be answered by exposing rodents throughout their life span to these compounds. Results of such studies would provide information about the carcinogenic potential of relatively weak mutagens.

Evaluation of the mutagenic and carcinogenic potential of chemotherapeutic agents must include consideration of pharmakinetic properties as well as of mutagenic properties of these compounds and their metabolites. Until a completely non-mutagenic antischistosomal agent has been developed, the mutagenic properties of available schistosomicides should be compared with each other so that the drug with the lowest mutagenic risk becomes recognizable. In fact, a WHO committee has recommended that "where there is a choice of several drugs of known mutagenic potential, the drug with the smallest risk for the individual and the population should be used" (WHO Reports, 1971). Yet, this organization has failed to apply such a rule to antischistosomal compounds (WHO Scientific Group, 1972).

There are very large differences in mutagenic activities of hycanthone and IA-4 N-oxide; yet in mice both compounds are equipotent as schistosomicides. Furthermore, the acute toxicity of IA-4 N-oxide is more than 12 times lower than that of hycanthone. Hence, structural modifications of the hycanthone molecule have reduced markedly both acute toxic and mutagenic properties, while desired chemotherapeutic activities have been maintained.

-52-

AMES, B.N., DURSTON, W.E., YAMASAKI, E. AND LEE, F.D.: Carcinogens

6. Experimentally Produced Resistance to Hycanthone in Schistosoma Mansoni

Genetically transferred resistance of Schistosoma mansoni to the anti-schistosomal drug hycanthone has been reported from this laboratory (A1). This type of drug resistance was observed in the progeny of mature worms that had been exposed to hycanthone within their host. Since we found subsequently that hycanthone resistance can occur also under different conditions (see below) the term Type I will be used to designate the drug resistant progeny of schistosomes that had been exposed to a single dose of hycanthone within their host after the worms had developed to the adult stage (at least 50 days after exposure of the host to cercariae). A second type of resistance (Type II) was observed in the progeny of worms whose hosts had been treated with hycanthone when these ancestors had been in an immature stage (27 to 30 days after cercarial exposure). The third type of hycanthone resistance (Type III) was found in the progeny of worms from hosts that had been infected with one sex of cercariae, followed by infections with the opposite sex, 2 to 22 weeks thereafter. Hence, Type III resistance did not involve exposure of the parental generation to hycanthone at any time.

Type I: Progeny of S. mansoni whose hosts were treated with hycanthone after maturation of the worms.

As illustrated in Fig. A1, this type of drug resistance has remained stable for 21 generations. The degree of resistance is expressed for each generation as the percentage of worms surviving 7 weeks after a dose of 80 mg/kg of hycanthone administered intramuscularly to the host. It should be noted that this approaches the maximum dose tolerated by mice infected with S. mansoni.

Type II: Progeny of S. mansoni whose hosts were challenged 27 to 29 days after cercarial exposure.

Hycanthone resistance has been observed in the progeny of worms that had been exposed to hycanthone at an immature stage. When mouse hosts were treated 27-29 days after infection with cercariae of S. mansoni with either 60 mg/kg or 3 mg/kg of hycanthone, and when thereafter the worms were allowed to mature they gave rise to a hycanthone-resistant progeny. The immature worms, being not susceptible to hycanthone, matured to the adult stage. These adults were susceptible to hycanthone when the hosts were challenged again with the drug. However, if not exposed to the drug for a second time, these worms gave rise to hycanthone-resistant progeny. The emergence of this type of resistance was restricted to the administration of the drug at a relatively short period during the development of the worms (i.e., 27 to 29 days following cercarial infection). Administration of hycanthone to the host after shorter or longer time intervals following exposure to cercariae produced resistant progeny only rarely. It should be noted that 27 to 29 days after infection of the host with cercariae both male and female reproductive systems are undergoing active development.

In five out of six experiments, administration of 60 mg/kg of hycanthone i.m. to the host 28 days after infection with a Puerto Rican (M) strain, resulted in resistant progeny. Several of these strains were followed for 16 generations and the resistance levels of one such genealogy is reproduced in Fig. A2. As in Type I, resistance was reduced in some generations (e.g., F_{13}), but increased subsequently.

A similar resistance was produced in 2 out of 3 experiments when a 20 times lower dose (3 mg/kg) of hycanthone was administered to mice 28 days after cercarial infection. In one of these, the resistance was maintained for 16 generations (Fig. A3).

The production of this type of resistance was not limited to a single strain (Puerto Rican M); it was observed also with another Puerto Rican strain (SW) and a St. Lucian strain. With the S.W. Puerto Rican strain, resistance was produced with 60 mg/kg in 2 out of 2 experiments (28 and 29 days after infection, respectively); in 2 out of 2 experiments with 16 mg/kg (28 to 30 days after infection), and in 4 out of 4 experiments with 3 mg/kg hycanthone (27, 28, 29, 30 days after infection, respectively). Resistance was followed for 9 generations and is reproduced in Fig. A4.

In yet another strain (from St. Lucia) the same type of resistance was produced in 9 out of 10 experiments, using 60 mg/kg i.m. of hycanthone, 27, 28, 29, 30 days after cercarial exposure, respectively. In two instances this resistance was followed for 11 generations (Fig. A5).

Using a similar procedure, only occasionally was a small degree of resistance observed with either 3 or 60 mg/kg with a Liberian strain of S. mansoni. With another Puerto Rican (WR) strain, no significant degree of resistance was induced under the same conditions.

Two progenies of hosts that had been treated for 28 days after cercarial exposure with another antischistosomal agent, oxamniquine (35 mg/kg i.m. or orally), were also found to be resistant to both hycanthone and oxamniquine.

Type III: Progeny of S. mansoni whose hosts had been infected with one sex of cercariae followed by infection with the opposite sex 2 to 22 weeks thereafter (Type III).

When mice were infected with only one sex of cercariae (either male or female) 13 to 133 days prior to infection with cercariae of the opposite sex, the progeny of the resulting adult worms was found to exhibit varying degrees of hycanthone resistance. Mice were first exposed to female cercariae of the Puerto Rican M strain, and subsequently, 106 days later, to male cercariae. The progeny of worms originating from such a sequential introduction of the two sexes was resistant to a high dose of hycanthone (80 mg/kg). This resistance has been maintained at high levels for 12 generations (Fig. A6). Varying degrees of drug resistance were observed also when the intervals between the introduction of the cercariae was longer or shorter. Whenever this was determined, resistance was found to fluctuate, but did not appear to be eliminated in the subsequent generations (F_2 to F_5).

With another Puerto Rican strain (SW), the progeny exhibited approximately 50% hycanthone resistance over 8 generations when the female cercariae giving rise to the original parent strain were introduced 107 days prior to the males.

When the sequence of infecting the hosts with the two sexes was reversed, i.e., when the male cercariae were introduced 106 days prior to the females, the progeny proved to be hycanthone-resistant also. In the F_1 generation, in three out of four experiments, resistance was complete (100%) and in the fourth a 50% level of resistance was observed. In subsequent generations, this resistance fluctuated to a much greater extent than when the female, rather than the male, cercariae had been introduced first. In the first eight generations, there was a tendency towards a progressive decline to a complete loss of resistance; however, this was followed by reappearance of a completely resistant F_9 generation and the maintenance of a high level of resistance in the next two generations (Fig. A7). The further course of this genetic transfer of resistance is currently being followed.

The degree of resistance varied considerably depending on the time interval which was allowed to elapse between introduction of male and female cercariae. For example, resistance to an extent of 45%, 31%, 26%, 31% was observed in F_1 when the female cercariae were introduced 46, 31, 144, 273 days after the male, respectively.

With this type of resistance (Type III), there was cross-resistance to two chloroindazole analogs of hycanthone, IA-3 and IA-4 (A2). IA-4 is equipotent with hycanthone against susceptible strains while IA-3 is slightly less active; yet a subcutaneous dose which is 7 times higher than the LD_{50} of hycanthone in the case of IA-4 (2 g/kg) and more than 10 times the LD_{50} of hycanthone for IA-3 (3 g/kg), complete resistance was observed in the F_2 progeny of the sequentially introduced cercariae, regardless of whether female or the male cercariae were introduced first. Both types of progeny remained susceptible to two nitroheterocyclic antischistosomal compounds, niridazole and SQ 18,506.

Genetically transferred drug resistance described in this report cannot be explained completely by selection of a preexisting resistant strain. For example, in Type II, immature worms gave rise to a hycanthone-resistant progeny, whereas the parents themselves remained susceptible. The possibility that hycanthone treatment resulted in the selection of preexistant hycanthone-resistant oocytes is not supported by the observation that the number of eggs produced by the adult worms was not reduced when 3 mg/kg had been administered 28 days after cercarial infection. Yet, under these conditions strong resistance was observed in the progeny of two strains of S. mansoni.

Some genetically transferred characteristic conferring hycanthone resistance in the progeny is produced by exposure of germ cells to hycanthone at a brief period during development of the parasite (Type II) or by sequential, rather than by simultaneous, introduction of the two sexes of cercariae into the host. Since hycanthone resistance was found to be maternally transferred, the possibility of extrachromosomal inheritance should be considered.

On the basis of our observations with five strains of S. mansoni, it is not possible to predict in how many strains any or all of the three types of resistance can be produced. However, Type II resistance could readily be produced in 3 out of 5 strains, and Type III in both of two strains, while Type I has been found so far to occur in only one.

It should be noted that this is the first reported instance of experimentally produced drug resistance in a parasitic metazoan that appears in the first generation (F_1) following a single exposure of the parent generation. In another helminth (Hemonchus contortus) drug resistance to thiabendazole is seen after progressive selection from a partially resistant population of worms.

It does not appear that the ability of the parasites to take up the drug or its metabolites had been altered. Recent observations in this laboratory indicate that after administration of a chloroindazole analog of hycanthone, IA-4, to which hycanthone-resistant worms are cross-resistant, the levels of this drug and of its metabolites are the same in hycanthone-susceptible and hycanthone-resistant schistosomes. Possibly some changes associated with pyrimethamine resistance of malarial parasites may be analogous, such as reduced binding of the drug or of its active metabolite, or the synthesis of an increased amount of an enzyme whose activity is inhibited by the drug (A3). In any case, as in the case of hycanthone resistance of schistosomes, pyrimethamine resistance has appeared after a single exposure of malarial parasites to the drug (A4-A6).

Some of the drug resistance observed in this study may have been carried over from an earlier stage of the life cycle of the parasite. It is well known that immature stages (schistosomulae) are not susceptible to hycanthone. This may be similar to a phenomenon observed with malarial parasites. The occurrence of chloroquine-resistant asexual forms of *P. falciparum* is contrasted by the lack of chloroquine resistance of the other three human malarial parasites. This may be related to the fact that the infectivity of the gametocytes of the latter three species is destroyed by chloroquine, while the sexual forms of *P. falciparum* are resistant to the drug.

Summary. Genetically transferred resistance to the antischistosomal drug hycanthone can be produced in some strains of *Schistosoma mansoni* (a) by administration of the drug to hosts of adult schistosomes (Type I), (b) by exposure of immature schistosomes to the drug within their host, 27 to 30 days after infection with cercariae (Type II), and (c) by infection of the host with one sex of cercariae followed by infection with the opposite sex 13 to 133 days thereafter (Type III).

References

A1. Rogers & Bueding: Science 172, 1057, 1971.

A2. Bueding, Fisher & Bruce: J. Pharmacol. Exp. Ther. 186, 402, 1973.

A3. Ferone: J. Biol. Chem. 245, 850, 1970.

A4. Avery-Jones: East. African Med. J. 31, 47, 1954.

A5. Young & Burgess: Bull. W.H.O. 20, 37, 1959.

A6. Young: Am. J. Trop. Med. Hyg. 6, 621, 1957.

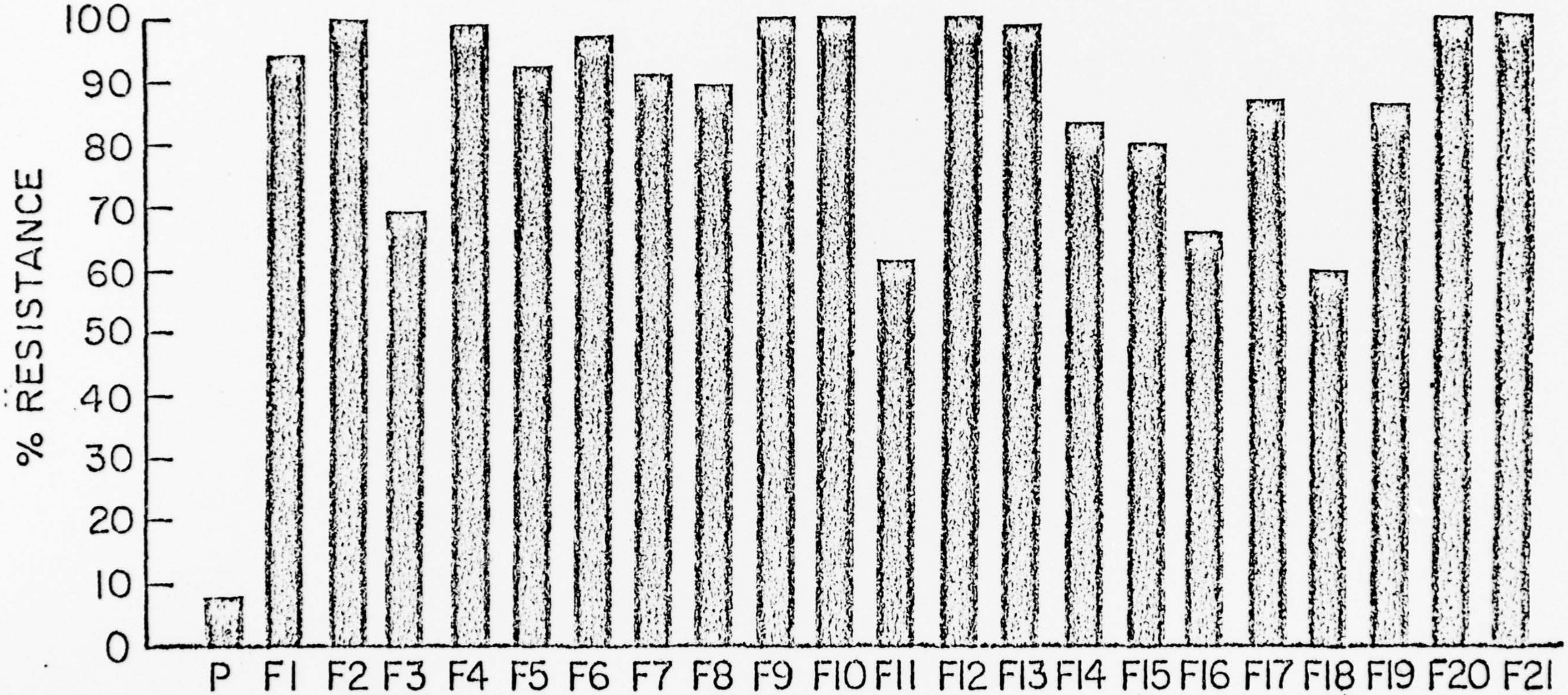


Fig. A1. Maintenance of hycanthone resistance (Type I) throughout 21 generations. Ordinate indicates percent of worms (Puerto Rican M strain) surviving challenge of the host with 80 mg/kg hycanthone (i.m.) 56 to 64 days after infection. P indicates parent strain.

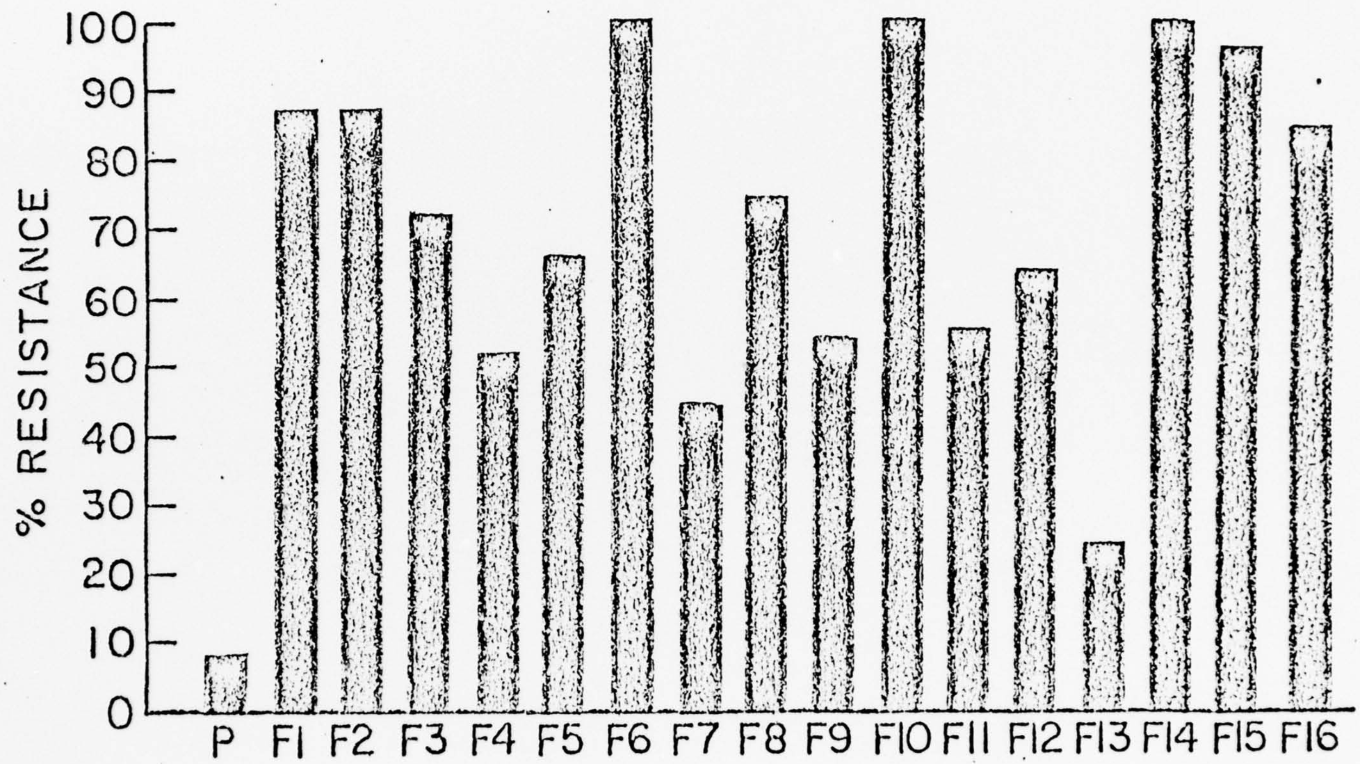


Fig. A2. Hycanthone resistance in progeny of parent schistosomes (Puerto Rican M strain) to whose hosts 60 mg/kg of hycanthone had been administered 28 days after cercarial exposure. Ordinate: percent resistance, P: parent strain.

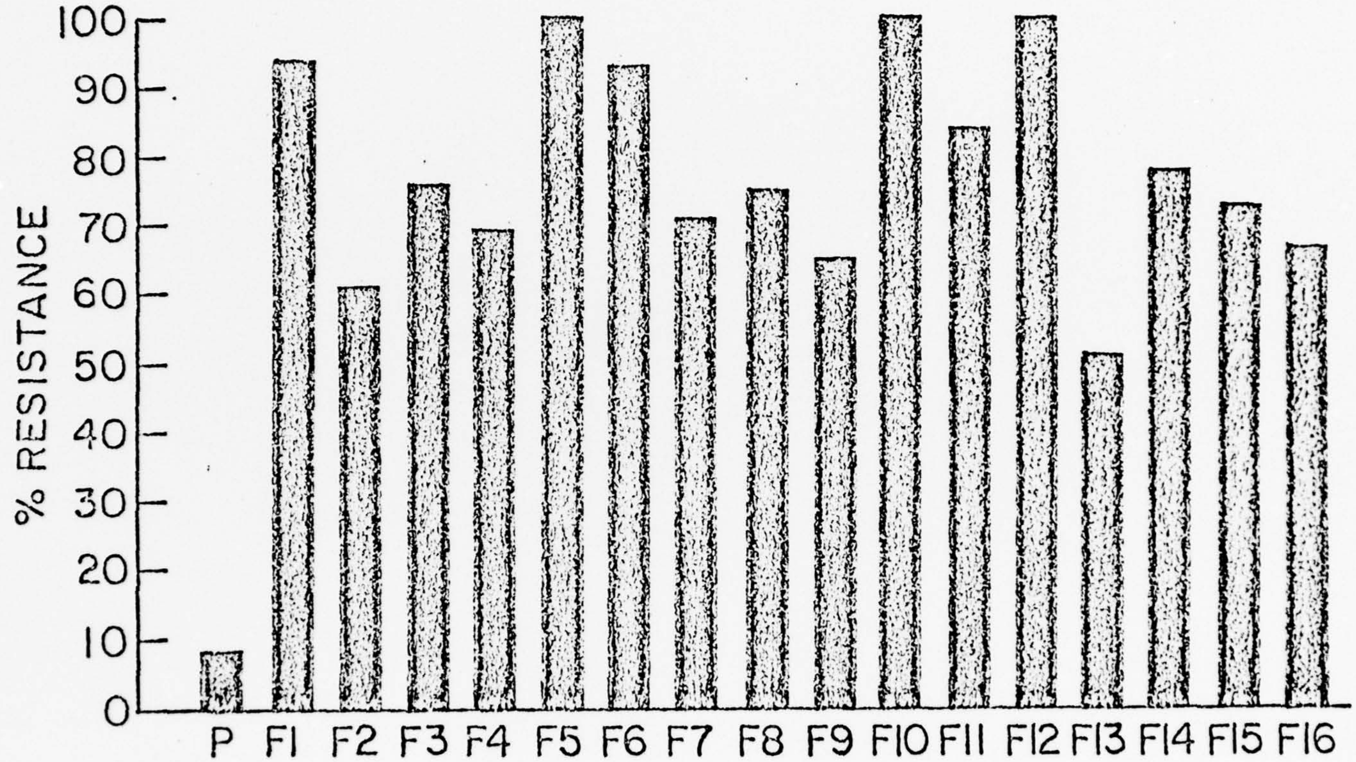


Fig. A3. Hycanthone resistance in progeny of parent schistosomes (Puerto Rican M strain) to whose hosts 3 mg/kg hycanthone had been administered (i.m.) 28 days after cercarial exposure. Ordinate: percent resistance, P: parent strain.

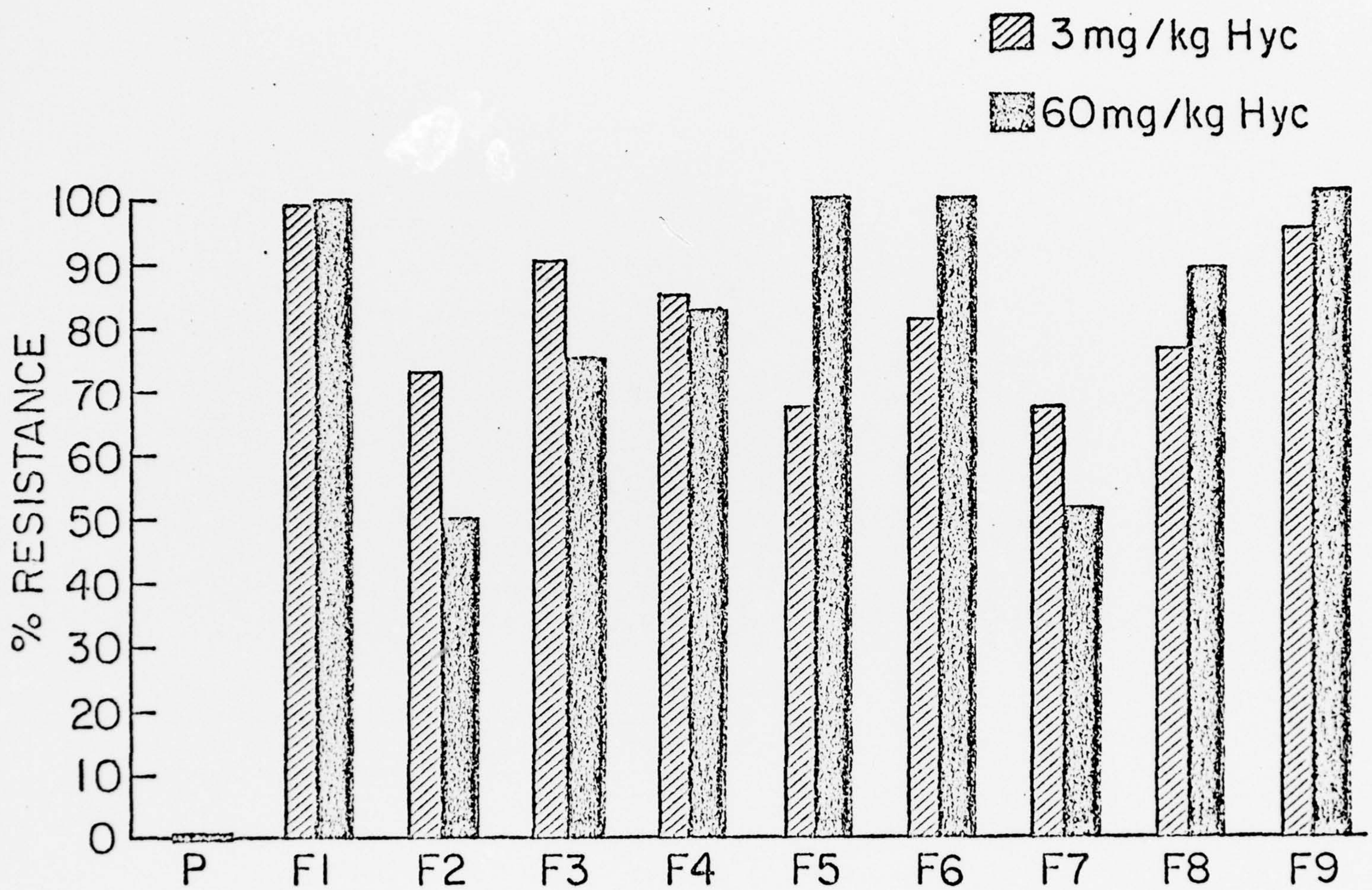


Fig. A4. Hycanthone resistance in progeny of Puerto Rican (S.W.) strain of parent schistosomes to whose hosts a low 3 mg/kg (shaded bars) or 60 mg/kg (black bars) of hycanthone had been administered (i.m.) 29 days after cercarial exposure. Ordinate: percent resistance, P: parent strain.

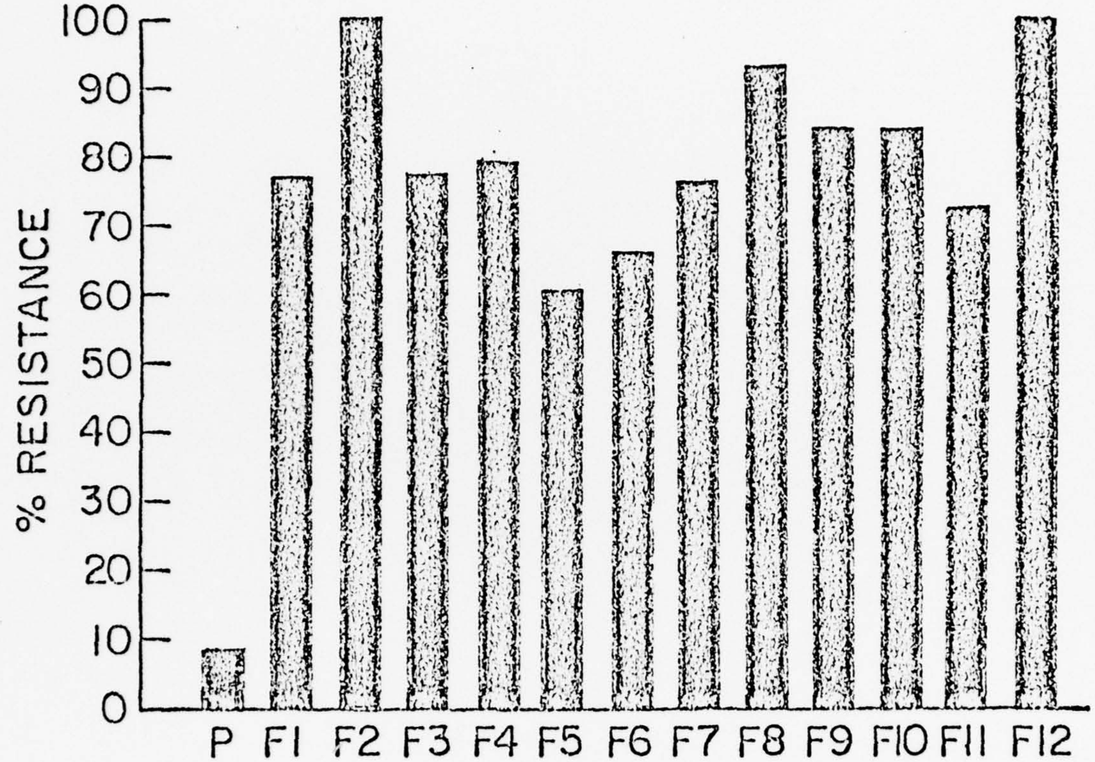


Fig. A5. Hycanthone resistance in progeny of parent schistosomes (St. Lucian strain) to whose host 60 mg/kg of hycanthone had been administered (i.m.) 27 days after cercarial exposure. Ordinate: percent resistance, P: parent strain.

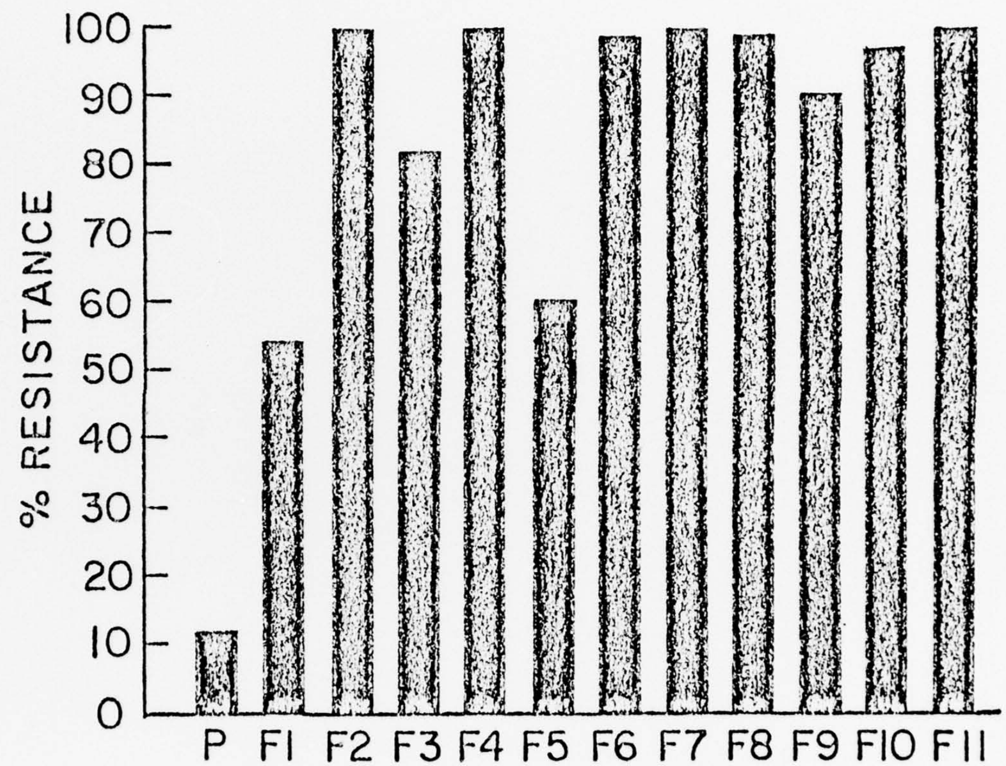


Fig. A6. Hycanthone resistance (Type III) in progeny of S. mansoni (Puerto Rican M strain) originating from sequentially introduced female cercariae, followed 106 days thereafter by male cercariae. Ordinate: percent resistance, P: parent strain.

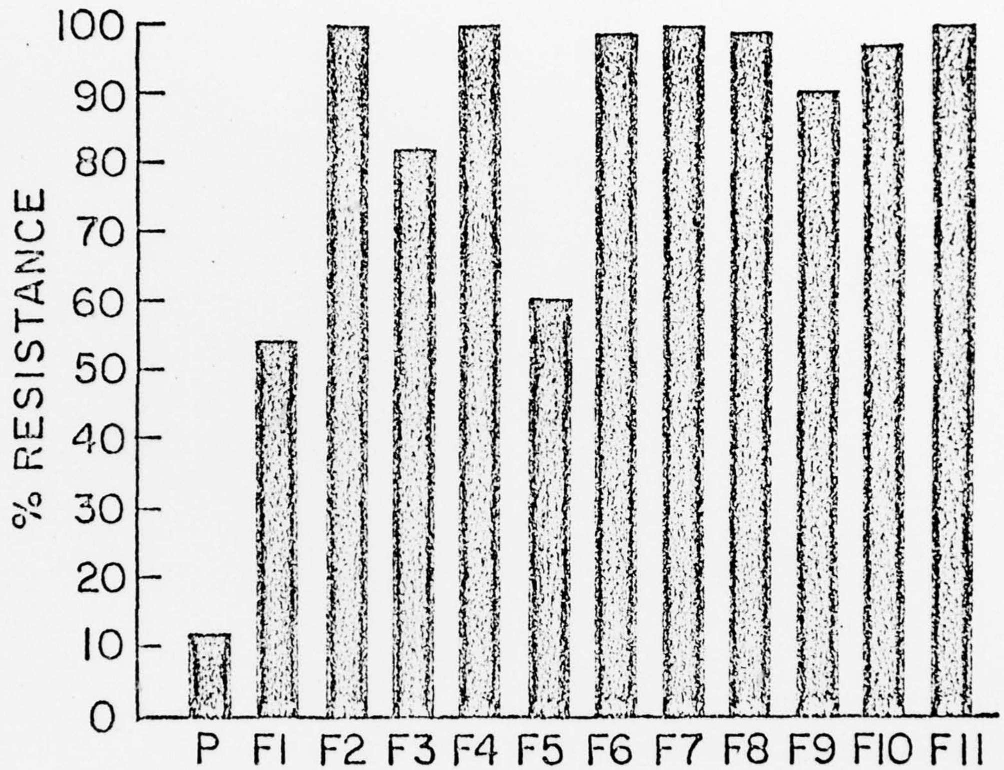


Fig. A6. Hycanthone resistance (Type III) in progeny of S. mansoni (Puerto Rican M strain) originating from sequentially introduced female cercariae, followed 106 days thereafter by male cercariae. Ordinate: percent resistance, P: parent strain.

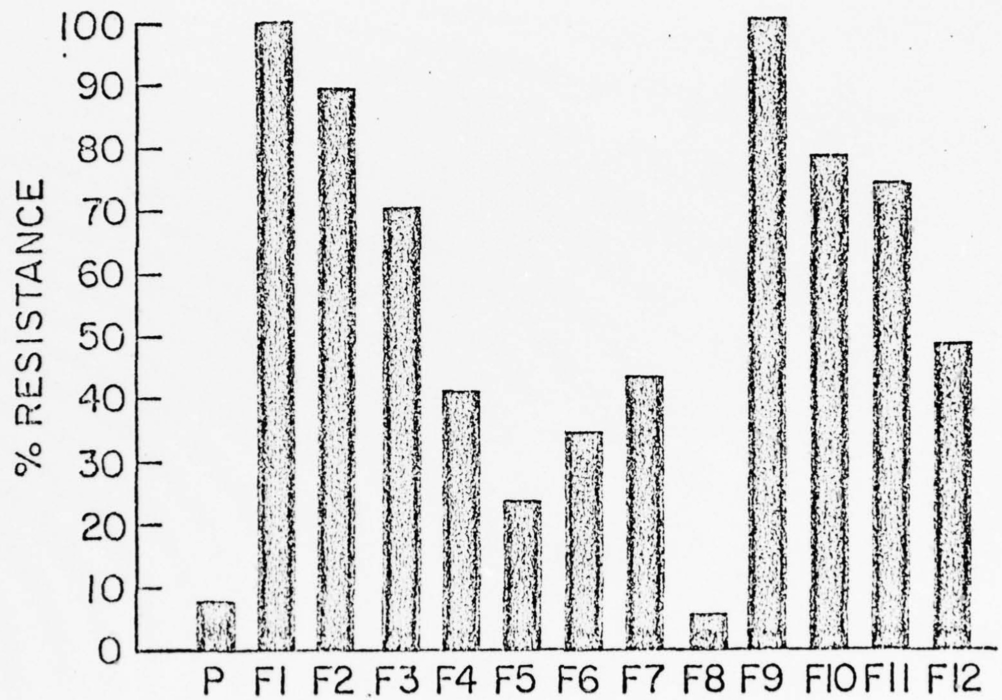


Fig. A7. Hycanthone resistance (Type III) i progeny of S. mansoni (Puerto Rican M strain) originating from sequentially introduced male cercariae followed 106 days thereafter by female cercariae.

AMES, B.N., DURSTON, W.E., YAMASAKI, E. AND LEE, F.D.: Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. (U.S.A.) 70: 2281-2285, 1973.

AMES, ~~B.A.~~ B.N., McCANN, J. AND YAMASAKI, E.: Methods for detecting carcinogens as mutagens with the Salmonella mammalian microsome mutagenicity test. Mutation Res. 31: 347-364, 1975.

BLAIR, D.M. AND WEBER, M.C.: Oral oxamniquine in the treatment of persistent *Schistosoma mansoni* bilharziasis. Centr. Afr. J. Med. 21: 24-26, 1975.

BUEDING, E.:Dissociation of mutagenic and other toxic properties from schistosomicides. J. Toxicol. Environ. Health. 1: 329-334, 1975.

BUEDING, E., FISHER, J. AND BRUCE, J.: The antischistosomal activity of a chloroindazole analog of hycanthone in mice infected with *Schistosoma mansoni*. J. Pharmacol. Exper. Ther. 186: 402-407, 1973.

CLIVE, D.: Mutagenicity of thioxanthenes (hycanthone, lucanthone and four indazole derivatives) at the TK locus in cultured mammalian cells. Mutation Res. 26: 307-318, 1974.

CLIVE, D., FLAMM, W.G. AND MACHESKO, M.R.:Mutagenicity of hycanthone in mammalian cells. Mutation Res. 14: 262-264, 1972.

COMMONER, B., VITHAYATHIL, A.J. AND HENRY, J.I.: Detection of metabolic carcinogen intermediates in urines of carcinogen-fed rats by means of bacterial mutagenesis. Nature (London) 249: 850-852, 1974.

da SILVA, L.C., SETTE, Jr., H., CHAMONE, D.A.F., SAEZ-ALQUEZAR, A., PUNSKAS, J.A. AND RAIA, S.: Clinical trials with oxamniquine (UK 4271) for the treatment of mansonian schistosomiasis. Rev. Inst. Med. Trop. Sao Paulo. 16: 103-109, 1974.

da SILVA, L.C., SETTE, Jr., H., CHAMONE, D.A.F., SAEZ-ALQUEZAR, A., PUNSKAS, J.A. AND RAIA, S.: Further clinical trials with oxamniquine (UK 4271), a new antischistosomal agent. Rev. Inst. Med. Trop. Sao Paulo. 17: 307-311, 1975.

DAVIS, A. AND BAILEY, D.R.: Metrifonate in urinary schistosomiasis. Bull. Wld. Health Org. 41: 209-224, 1969.

DRAKE, J.W.:(Chairman of Committee 17 report, Environmental Mutagen Society), Environmental mutagenic hazards. Science 187: 503-514, 1975.

DURSTON, W.E. AND AMES, B.N.: A simple method for the detection of mutagens in urine: studies with the carcinogen 2-acetylaminofluorene. Proc. Natl. Acad. Sci. (U.S.A.) 71: 737-741, 1974.

ELSLAGER, E.F.: Schistosomiasis chemotherapy: Progress at a snail's pace. In Abstracts of Papers, 12th National Medicinal Chemistry Symposium of the American Chemical Society, Seattle p. 9g., June 22-25, 1970.

FORSYTH, D.M. AND RASHID, C.: Treatment of urinary schistosomiasis, Lancet 1: 130-133, 1967.

Treatment of Urinary schistosomiasis with trichlorophone. Lancet 2: 909-912, 1967.

Insert ref.) GENTILINI, M., DAVIS, M., HOUENASSCU, P. AND ARNAUD, J.P.: Résultat de l'activité schistosomicide, d'un organo-phosphoré, le métrifonate, dans la bilharziose urinaire, Bull. de la Société de Pathologie Exotique. 66: 299-306, 1973.

GREEN, S., CARR, J.V., SAURO, F P.M. AND LEGATOR, M.S.: Effects of hycanthone on spermatagonial cells, deoxyribonucleic acid synthesis in bone marrow and dominate lethality in rats. J. Pharmacol. Exp. Ther. 187: 437-443, 1973a.

GREEN, S., SAURO, F.M. AND LEGATOR, M.S.: Cytogenetic effects of hycanthone in the rat. Mutation Res. 17: 239-244, 1973b.

HAESE, W.H., SMITH D.L. AND BUEDING, E.: Hycanthone-induced hepatic changes in mice infected with Schistosoma mansoni. J. Pharmacol. Exp. Ther. 186: 430-440, 1973.

HAESE, W.H. AND BUEDING, E.: Long-term hepatocellular effects of hycanthone and of two other antischistosomal drugs in mice infected with Schistosoma mansoni. J. Pharmacol. Exp. Ther. ~~in press~~, 197: 703-713, 1976.

HANNA, S., BASMY, K., SELIM, O., SHOEB, S.M. AND AWNY, A.Y.: Effects of administration of an organophosphorus compound as an anti-bilharzial agent, with special reference to plasma cholinesterase. British Med. J. 1: 1390-1392, 1966.

GABRIDGE, M.G., DENUNZIO, A. AND LEGATOR, M.S.: Microbial mutagenicity of streptozotocin in animal-mediated assays. Nature 221: 68-71, 1969.

HARTMAN, P.E., BERGER, H. AND HARTMAN, Z.: Comparison of hycanthone ('Etrenol'), some hycanthone analogs, myxin and 5-nitroquinoline-1-oxide as frameshift mutagens. J. Pharmacol. Exp. Ther. 186: 390-398, 1973.

HARTMAN, P.E. AND HULBERT, P.B.: Genetic activity spectra of some antischistosomal compounds, with particular emphasis on thioxanthenones and benzothiopyranindazoles. J. Toxicol. Env. Health 1: 243-270, 1975.

HARTMAN, P.E., HULBERT, P.B., BUEDING, E. AND TAYLOR, D.D.: Microsomal activation to mutagens of antischistosomal methyl thioxanthones and initial tests on a possibly non-mutagenic analogue. Mutation Res. 31: 87-95, 1975.

HARTMAN, P.E., LEVINE, K., HARTMAN, Z. AND BERGER, H.: Hycanthone: A frameshift mutagen. Science 172: 1058-1060, 1971.

HETRICK, F.M. AND KOS, W.L.: Transformations of Rauscher virus-infected cell cultures after treatment with hycanthone and lucanthone. J. Pharmacol. Exp. Ther. 186: 425-429, 1973.

HULBERT, P.B., BUEDING, E. AND HARTMAN, P.E.: Hycanthone analogs: Dissociation of mutagenic effects from antischistosomal effects. Science 186: 647-648, 1974.

KATZ, N., GRINBAUM, E., CHAVES, A., ZICKER, F. AND PELLEGRINO, J.: Clinical trials with oxamniquine, by oral route, in schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo In Press, (1976).

KNAAP, A.G.A.C. AND KRAMERS, P.G.N.: Mutagenic effects of hycanthone in Drosophila melanogaster. Mutation Res. 21: 38-39, 1973.

KOURA, M., GABER, A. ABDEL-MEGUID, M. AND SAIF, M.: Oxamniquine in the treatment of S. mansoni infection in Egypt. Abstr. Internatl. Conf. on Schistosomiasis, Cairo, Egypt, p.68, Oct, 1975.

KRAMERS, P.G. AND KNAAP, A.G.A.C.: Low mutagenic activity of four hycanthone analogues in Drosophila melanogaster. Mutation Res. 31: 395-400, 1975.

LEE, S.Y.: Current status of the host-mediated L5178Y system. Environ. Health Perspec 6: 145-149, 1973.

LEGATOR, M.S. AND MALLING, H.V.: The host-mediated assay, a practical procedure for evaluating potential mutagenic agents in mammals. In A. HOLLAENDER (Ed.), Chemical Mutagens. Principles and Methods for their Detection. Vol. 2, p. 569-589, Plenum, New York, 1971.

LUCIER, G.W., McDANIEL, O.S., BEND, J.R. AND FAEDER, E.: Effects of hycanthone and two of its chlorinated analogs on hepatic microsomes. J. Pharmacol. Exp. Ther. 186: 416-424, 1973.

MALLING, H.V.: Dimethylnitrosamine: formation of mutagenic compounds by interaction with mouse liver microsomes. Mutation Res. 13: 425-429, 1971.

McCANN, J. AND AMES, B.N.: Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. Proc. Natl. Acad. Sci. (U.S.A.) 73: 950-954, 1976.

McCANN, J., CHOI, E. YAMASAKI, E. AND AMES, B.N.: Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci (U.S.A.) 72: 5135-5139, 1975a.

McCANN, J., SPINGARN, N.E., KOBORI, J. AND AMES, B.N.: Detection of carcinogens as mutagens: Bacterial tester strains with R Factor Plasmids. Proc. Natl. Acad. Sci. (U.S.A.) 72: 979-983, 1975b.

MEADOW, M.G., QUAH, S.-K. AND von BORSTEL, R.C.: Mutagenic action of hycanthone and IA-4 on yeast. J. Pharmacol. Exp. Ther. 187: 444-450, 1973.

MOORE, J.A.: Teratogenicity of hycanthone in mice. Nature 239: 107-109, 1972.

NIEMANDT, S. AND MURAHWA, S.: Metrifonate in the treatment of bilharziasis. S. Afr. Med. J. 49: 1860, 1975.

ONGOM, V.L., WAMBOKA, G.W. AND KADIL, A.K.: Oxamniquine (UK 4271) in the treatment of *S. mansoni* infections in Uganda. Abstr. Internatl. Conf. on Schistosomiasis, Cairo, Egypt, p.69, Oct. 1975.

RAY, V., HOLDER, H.E., ELLIS, Jr., J.H. AND HYNECK, M.L.: A comparative study of the genetic effects of hycanthone and oxamniquine. J. Toxicol. Env. Health 1: 211-227, 1975.

REDDY, S., OOMEN, J.M.V. AND BELL, D.R.: Metrifonate in urinary schistosomiasis: A field trial in northern Nigeria. Ann Trop. Med. and Parasitol. 69: 73-76, 1975.

SARMA, D.S.R., RAJELAKSHMI, S. AND ZUBROFF, J.: *In vivo* repair of liver DNA damaged by hycanthone and related compounds. Molec. Pharmacol. in press, (1976) SAURO, F.M. AND GREEN, S.: *In vivo* cytogenetic evaluation of chloroindazole thioxanthene IA-4 (a hycanthone analog) and niridazole in rat bone marrow. J. Pharmacol. Exp. Ther. 186: 399-401, 1973.

SIONGOK, T.K.A., OUMA, J.H. AND KABIRU, J.: Report on the treatment of *Schistosoma mansoni* infection in school children with oxamniquine. Abstr. Internatl. Conf. on Schistosomiasis, Cairo, Egypt, p.66-68, Oct. 1975.

VOGEL, H.J. AND BONNER, D.M.: Acethylornithinase of *E. coli*: partial purification and some properties. J. Biol. Chem. 218: 97-106, 1956.

von BORSTEL, R.C. AND QUAH, S.-K.: Induction of mutations in saccharomyces with hycanthone. Mutation Res. 21: 52, 1973.

WEISBURGER, J. AND WEISBURGER, E.: Tests for chemical carcinogens, In H. BUSCH (Ed.). Methods in Cancer Research, Vol. 1, p. 306-399, Academic Press, New York, 1967.

WHO Reports on Schistosomicidal Drugs, Bull. Offic. Sanit. Panamer. Engl. Ed. 6: 82-100, 1972

WHO Scientific Group, Evaluation and Testing of Drugs for Mutagenicity: Principles and Problems. WHO Techn. Rep. Ser. No. 482, 1971

FOOTNOTE

1) T. Sugimura: Personal communication.

DISTRIBUTION LIST

4 copies	HQDA (SGRD-RP) WASH DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014